

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

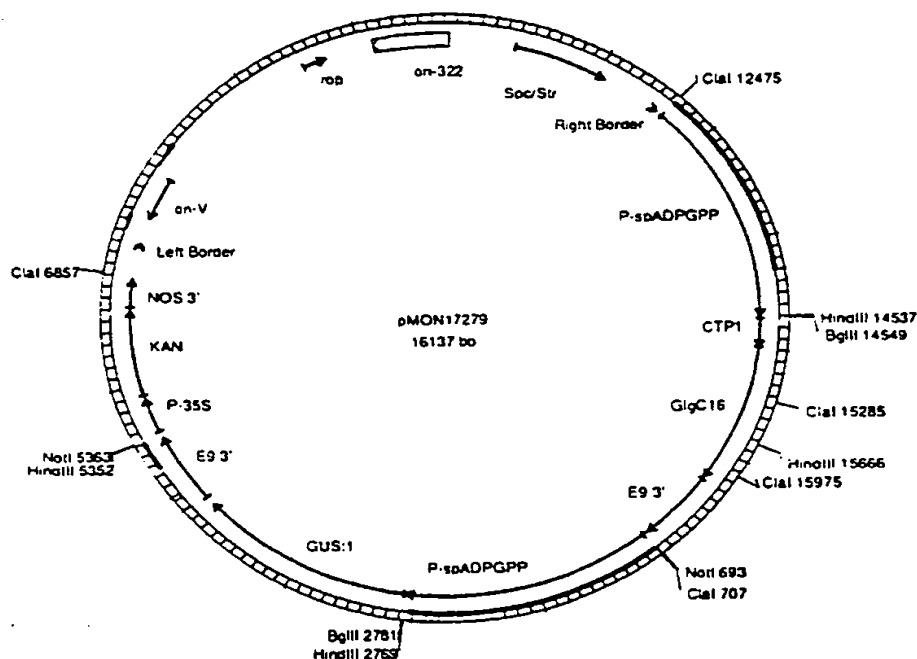


#8

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/82, 15/54, 5/10, A01H 5/00		A1	(11) International Publication Number: WO 94/28149
			(43) International Publication Date: 8 December 1994 (08.12.94)
(21) International Application Number: PCT/US94/05275		(74) Agent: BOLDING, James, Clifton; Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).	
(22) International Filing Date: 18 May 1994 (18.05.94)		(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FL, GE, HU, JP, KG, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(30) Priority Data: 070,155 28 May 1993 (28.05.93) US			
(60) Parent Application or Grant (63) Related by Continuation US 070,155 (CIP) Filed on 28 May 1993 (28.05.93)		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant (for all designated States except US): MONSANTO COMPANY [US/US]; 800 North Lindbergh Boulevard, Saint Louis, MO 63167 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): BARRY, Gerard, Francis [IE/US]; 6350 Waterman Avenue, St. Louis, MO 63130 (US). KISHORE, Ganesh, Murthy [US/US]; 5354 Grantley Drive, Chesterfield, MO 63017 (US). STARK, David, Martin [US/US]; 1218 Summerpoint Lane, Fenton, MO 63026 (US). ZALEWSKI, James, Conrad [US/US]; 3009 East Nature Drive, Boise, ID 83716 (US).			

(54) Title: METHOD OF IMPROVING THE QUALITY OF STORED POTATOES



(57) Abstract

A method of improving the quality of potatoes stored at reduced temperatures and a method of prolonging dormancy of stored potato tuber by increasing the level of ADPglucose pyrophosphorylase enzyme activity within the potato tuber during storage at ambient or reduced temperatures. Novel DNA molecules, plant cells, and potato plants are provided wherein the gene for the ADPglucose pyrophosphorylase enzyme is under the control of a cold-inducible promoter.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

METHOD OF IMPROVING THE QUALITY OF STORED POTATOES

Long term storage properties of potato represents a major determinant of tuber quality. Dormancy periods (the time period after harvesting and before 5 sprouting) are crucial to maintaining quality potatoes. Commercially, potatoes may be held for long periods before processing (up to 10 months or longer), and at temperatures typically between 2-10 °C. Cold storage (2-6 °C) versus storage at 7-12 °C provides the best long term conditions by reducing respiration, water loss, microbial infection, and the need for chemical sprout inhibitors 10 (Burton, 1989). However, low temperatures lead to cold-induced sweetening, and the resultant high sugar levels contribute to an unacceptable brown color in the fried product (Coffin et al., 1987, Weaver et al., 1978). The sugars that accumulate are predominantly glucose, fructose, and sucrose, and it is mainly the reducing sugars (primarily glucose and fructose) which react with free 15 amino groups upon heating during the various cooking processes, including frying, via the Maillard reaction, and result in the formation of brown pigment (Burton, 1989, Shallenberger et al., 1959). Sucrose, on the other hand, produces a black coloration on frying due to its susceptibility to undergo carmelization as well as charring. Levels of reducing sugars above 0.2% fresh weight 20 are sufficient to cause brown pigment formation and thus merit rejection for certain types of processing. A potato processor can reduce the levels of sugars by a costly and time consuming blanching process if the levels of sugars are not significantly higher than the 0.2% limit. Potatoes can be reconditioned at higher temperatures (18 °C) to lower sugar content, but often sugar levels will 25 not sufficiently decrease before the onset of sprouting at these temperatures, requiring the use of chemical sprout inhibitors (Ryall and Lipton, 1979, Hardenburg et al., 1986). However, reconditioning increases the storage facility requirements and consequently affects the final cost of the product. Furthermore, it has been shown that reconditioning is not effective after longer storage 30 periods (Coffin et al., 1987). Given the negative environmental and health perceptions associated with excessive chemical use, and the fact that current sprout inhibitors may soon be banned, a need exists for potato varieties which can withstand long term cold storage without the use of chemicals, without accumulation of reducing sugars, and with greater retention of starch levels.

35 After longer storage periods, sprouting of potato tubers becomes a

probl m. Excess sprouting reduces the market value and can cause increased levels of alkaloids in the tuber.

Through the process of genetic engineering potato tubers which contain significantly higher levels of starch have been obtained. See WO 91/19806 5 (Kishore), also U.S.S.N. 07/709,663, filed 6/7/91, hereby incorporated by reference. In these tubers a gene is expressed which encodes ADPglucose pyrophosphorylase (ADPGPP), which catalyzes a key step in starch and glycogen biosynthesis. The preferred gene is from *E. coli* and the resulting enzyme is a poorly regulated, highly active variant. When a mutant of this gene, *glgC16*, is 10 expressed in a tuber-specific manner, for example from a class I patatin promoter, starch levels are higher than those of nontransgenic control tubers at the time of harvest.

Carbohydrate metabolism is a complex process in plant cells. Manipulation of a number of different enzymatic processes potentially may effect the 15 accumulation of reducing sugars during cold storage. For example, sugars may be used to resynthesize starch, and thus effect reduction in the pool of free sugar. Other methods may also serve to enhance the cold storage properties of potato through reduction of sugar content, including the inhibition of starch hydrolysis, removal of sugars through glycolysis, or conversion of sugars into 20 other forms which would not participate in the Maillard reaction. The challenge in these methods would be to identify an activity with which to effect the desired result, achieve function at low temperatures, and still retain the product qualities desired by potato growers, processors, and consumers.

It has been suggested that phosphofructokinase (PFK) plays an 25 important role in the cold-induced sweetening process (Kruger and Hammond, 1988, ap Rees et al., 1988, Dixon et al., 1981, Claassen et al., 1991). ap Rees et al. (1988) suggested that cold treatment had a disproportionate effect on different pathways in carbohydrate metabolism in that glycolysis was more severely reduced due to the cold-lability of PFK. The reduction in PFK activity 30 would then lead to an increased availability of hexose-phosphates for sucrose production. Additional support for this view comes from the observation of a new breeding clone of potato which contains a PFK which is not cold labile and that does not accumulate significant amounts of sugar in the cold.

It was recently disclosed in European Patent Application 0 438 904 that 35 increasing PFK activity reduces sugar accumulation during storage by

removing hexoses through glycolysis and further metabolism. A PFK enzyme from *E. coli* was expressed in potato tubers and the report claimed to increase PFK activity and to reduce sucrose content in tubers assayed at harvest. However, it has been shown that pyrophosphate:Fructose 6-phosphate phosphotransferase (PFP) remains active at low temperatures (Claassen et al., 1991). PFP activity can supply fructose 6-phosphate for glycolysis just as PFK can since the two enzymes catalyze the same reaction. Therefore the efficacy of this approach in improving the cold storage quality of potato tubers remains in doubt. Furthermore, the removal of sugars through glycolysis and further metabolism would not be a preferred method of enhancing storage properties of potato tubers because of the resultant loss of valuable dry matter content through respiration. Resynthesis of the sugars into starch or slowing the breakdown of starch would be preferred because dry matter would be retained.

It is an object of this invention to provide a method for reducing the level of sugars within potato tubers and to provide improved quality of stored potatoes. It is a further object of this invention to provide potatoes having an improved rate and degree of reconditioning after storage at reduced temperatures. It is a still further object of this invention to provide a method of extending dormancy of potatoes stored at ambient temperatures or at reduced temperatures.

SUMMARY OF THE INVENTION

The present invention provides a method of improving the quality of potatoes stored at low temperatures comprising providing an increased level of ADPglucose pyrophosphorylase (ADPGPP) enzyme activity within the potato tuber during storage at reduced temperatures. Also provided is a method of reducing the level of sugars within potato tubers stored at reduced temperatures by increasing the ADPGPP enzyme activity during cold storage. Further provided is a method of prolonging dormancy of stored potatoes comprising increasing the ADPGPP enzyme activity during storage.

30 This method is preferably accomplished by:

(a) inserting into the genome of a potato plant cell a recombinant, double-stranded DNA molecule comprising

(i) a promoter which functions in plants to cause the production of an RNA sequence in target plant tissues,

35 (ii) a structural DNA sequence that causes the production of an RNA

sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an ADPglucose pyrophosphorylase enzyme,

(iii) a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence;

(b) obtaining transformed plant cells; and

(c) regenerating from the transformed plant cells genetically transformed potato plants which have improved cold storage properties.

Novel recombinant DNA molecules, plant cells, and regenerated potato plants are provided wherein the promoter of (a)(i) is a cold-inducible promoter, such as from potato or *Arabidopsis*. These regenerated potato plants are useful in all of the methods of the present invention.

A preferred ADPglucose pyrophosphorylase (ADPGPP) enzyme is that from *E. coli*, known as *glgC*, which gene sequence is shown below as SEQ ID NO:1 and which amino acid sequence is shown as SEQ ID NO:2. A more preferred ADPGPP enzyme is the mutant ADPGPP, *glgC16*, which gene sequence is shown below as SEQ ID NO:3 and which amino acid sequence is shown below as SEQ ID NO:4. This mutant has been found to have a higher affinity to substrates in the absence of the activator, fructose 1,6-bisphosphate (FBP), and to reach half-maximal activation with a decreased concentration of FBP.

As used herein, the term "improving the quality of stored potatoes," or variants thereof, shall mean providing potatoes which after storage have reduced levels of sugars, little or no loss of starch, reduced incidence of sprouting, and/or an enhanced rate or degree of reconditioning.

As used herein, the term "cold storage" or "storage at reduced temperature," or variants thereof, shall mean holding at temperatures less than or equal to 15 °C, which may be caused by refrigeration or ambient temperatures.

As used herein, the term "cold-inducible promoter" shall mean a sequence of DNA bases that initiates the transcription of mRNA using one of the DNA strands as a template to make a corresponding complimentary strand of RNA when the temperature is equal to or less than 15 °C.

As used herein, the term "prolonging dormancy" or variants thereof shall mean delaying onset of respiration and sprouting of tubers.

As used herein, the term "*glgC16* potatoes," "*glgC16* tubers," "*glgC16*

lines," or variants thereof, shall mean potato lines or tubers therefrom which have been transformed with a fusion of a plastid terminal transit peptide, preferably CTP, described below.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 shows a plasmid map for plant transformation vector pMON17316.

Figure 2 shows a plasmid map for plant transformation vector pMON17279.

DETAILED DESCRIPTION OF THE INVENTION

10 Starch phosphorylase and amylolytic enzymes are responsible for starch degradation during cold storage and result in the formation of glucose 1-phosphate and/or glucose from starch. Glucose may be converted to glucose 1-phosphate and serve as a substrate for the ADPGPP enzyme and thus for starch biosynthesis in the tubers expressing this enzyme. Glucose 1-phosphate may also be formed from the products of degradation of sucrose via
15 invertase or sucrose synthase. Reducing sugars primarily accumulate during storage rather than sucrose due to the action of invertase (Pressey, 1966). The glucose and fructose released from invertase activity can also serve as precursors of substrates for starch biosynthesis.

20 The expression of ADPGPP is an effective means of countering the effects of cold-induced sweetening. It is hypothesized that by maintaining starch biosynthesis during cold storage, the continuous demand on the hexose pool is such that sugar accumulation is reduced and thus the tuber remains suitable for processing. However, other mechanisms may also be responsible
25 for this effect of ADPGPP. In addition, prolonging the dormancy of potatoes stored at any temperature may also be accomplished by keeping the sugar level low and delaying onset of respiration and thus sprouting.

In order to accomplish the foregoing, a gene for expression of ADPGPP is incorporated within the genome of potato plants. This gene may be combined
30 with other genes (in sense or antisense orientation) for regulation of starch and/or sugar metabolism/catabolism in potatoes, for example, phosphofructokinases (EP 0 438 904); α - and β -amylases; sucrose phosphate synthases; hexokinases; starch phosphorylases; debranching enzymes; or phosphoglucotases. These additional genes may be from a plant, microorganism, or
35 animal source.

Alternatively, increased levels of ADPGPP in stored tubers may be achieved by mutagenizing potato clones and thus increasing ADPGPP enzyme activity levels. Such tubers could be selected based on display of increased specific activity, increased V_{max} , reduced inhibition by the negative effector (P_i), 5 or reduced dependence upon activator (3-PGA) for maximal activity.

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' nontrans-
10 lated region which adds polyadenylate nucleotides to the 3' end of the RNA.

Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA, and to initiate the transcription of mRNA using one of the DNA strands as a template to
15 make a corresponding complimentary strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as
20 the cauliflower mosaic virus (CaMV) 19S and 35S and the figwort mosaic virus 35S-promoters, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide), and the chlorophyll a/b binding protein gene promoter, etc. All of these promoters have been used to create various types of DNA constructs which
25 have been expressed in plants; see, e.g., PCT publication WO 84/02913.

The class I patatin promoters used in Examples 1 and 2 below, have been shown to be both highly active and tuber-specific (Bevan et al., 1986; Jefferson et al., 1990). A number of other genes with tuber-specific or enhanced expression are known, including the potato tuber ADPGPP genes, large and small
30 subunits (Muller et al., 1990), sucrose synthase (Salanoubat and Belliard, 1987, 1989), the major tuber proteins including the 22 kd protein complexes and proteinase inhibitors (Hannapel, 1990), the granule bound starch synthase gene (GBSS) (Rohde et al., 1990), and the other class I and II patatins (Rocha-Sosa et al., 1989; Mignery et al., 1988). Other promoters which are contem-
35 plated to be useful in this invention include those that show enhanced or

specific expression in potato tubers, that are promoters normally associated with the expression of starch biosynthetic or modification enzyme genes, or that show different patterns of expression within the potato tuber, with cortex- or pith- or periderm-enhanced expression, for example, or are expressed at 5 different times during tuber development. Examples of these promoters include those for the genes for the granule-bound and other starch synthases, the branching enzymes (Kossmann et al., 1991; Blennow, A. and Johansson, G., 1991; WO 92/14827; WO 92/11375), disproportionating enzyme (Takaha et al., 1993), debranching enzymes, amylases, starch phosphorylases (Nakano et al., 1989; Mori et al., 1991), pectin esterases (Ebbelaar, et al., 1993), the 40 kD glycoprotein; ubiquitin, aspartic proteinase inhibitor (Stukerlj et al., 1990), the carboxypeptidase inhibitor, tuber polyphenol oxidases (Shahar et al., 1992; GenBank® Accession Numbers M95196 and M95197), putative trypsin inhibitor and other tuber cDNAs (Stiekema et al., 1988), and for β -amylase and 15 sporamins (from *Ipomoea batatas*; Yoshida et al., 1992; Ohta et al., 1991).

Expression of bacterial ADPGPP from various potato promoters has been shown by Kishore in PCT Application WO 91/19806 to result in an increase in starch content in potato tubers.

It is not a requirement of the present method to start with a tuber with 20 high starch content to achieve low reducing sugar accumulation during cold storage. The *glgC16* gene can be expressed from a cold-induced promoter in potato so that the GlgC16 enzyme is only present during storage conditions. The presence of this enzyme would then maintain starch biosynthesis during storage and thus prevent the accumulation of sugars.

25 Examples of cold-inducible promoters, including plant promoters are numerous (Yamaguchi-Shinozaki et al., 1993; Qoronfleh et al., 1992; Miner et al., 1992; Houde et al., 1992; White et al., 1992; Huang et al., 1987; Murata et al., 1992; Gilmour et al., 1992, Hajela et al., 1990; and Kurkela et al., 1990). Isolation of cold-induced proteins in potato tubers has been demonstrated (van 30 Berkel et al., 1991, van Berkel et al., 1994). The promoters driving cold-induced expression of these proteins can be isolated by methods available to those skilled in the art. One method involves production of a cDNA library from cold stressed tubers and subsequent identification of cold-specific clones by differential hybridization with a non-stressed library. This process can be made more 35 efficient by using subtraction libraries wherein clones expressed in a non-cold-

specific manner are removed from the library during construction. The determination of the nucleotide sequences of cDNA's derived from these regulated transcripts will also facilitate the isolation of the corresponding promoter regions. The sequences of such cDNAs are known for a number of the potato tuber cold regulated transcripts (van Berkel et al., 1994). The promoter fragment could then be identified from a genomic clone using cDNA probes identified as cold-specific. Such cold regulated promoters have been identified and sequenced (Yamaguchi-Shinozaki and Shinozaki, 1994, and Baker, 1994). The promoter fragment can be used to direct expression of the *E. coli glgC16* gene in a cold-induced manner. Additionally, one of several other ADPGPP enzymes could be expressed from this promoter to affect sugar concentration in cold stored potato tubers and thus improve the quality of the tubers. Hybrid promoters or fusions of regulatory elements of different promoters may also be employed to increase the expression level of a cold regulated promoter or to make such expression more specific to the desired plant organ. Cold regulated genes have been described in which the expression is preferential in different tissues (Zhu et al., 1993) or in which the genes are regulated more specifically by cold than by other stress effects (Wilhelm and Thomashow, 1993; Nordin et al., 1993). In addition, specific defined sequences, of sizes from 9 base pairs to a few hundred base pairs in length, have been shown to control the responsiveness of the promoters to different cold and to other stress effects such as abscisic acid levels and to drought stress (Yamaguchi-Shinozaki and Shinozaki, 1994). Promoters that express preferentially in tubers are known and the regions of these promoters that are necessary for this preferential expression have also been determined (Jefferson et al., 1990; Liu et al., 1990). These data enable the construction of fusions between the small cold responsive element from promoters such as those from *cor78*, *cor 15a*, or *cor15b*, for example, and a patatin promoter. Fusions are made to the -500 to -2000 bp region of the patatin promoter. Current molecular genetic techniques, including Polymerase Chain Reaction and site-directed mutagenesis, and the facility of oligonucleotide synthesis make these fusions possible.

The amino-terminal plastid transit peptide used with the ADPGPP gene is needed to transport the enzyme to the plastid where starch synthesis occurs. Alternatively, the transit peptide could be omitted and the gene could be inserted into the DNA present in the plastid. Chloroplast transformation may

be accomplished using the methods described by Svab et al., 1990.

Production of Altered ADPglucose Pyrophosphorylase Genes by Mutagenesis

Those skilled in the art will recognize that while not absolutely required, enhanced results are to be obtained by using ADPglucose pyrophosphorylase genes which are subject to reduced allosteric regulation ("deregulated") and more preferably not subject to significant levels of allosteric regulation ("unregulated") while maintaining adequate catalytic activity. The structural coding sequence for a bacterial or plant ADPglucose pyrophosphorylase enzyme can be mutagenized in *E. coli* or another suitable host and screened for increased glycogen production as described for the *glgC16* gene of *E. coli*. It should be realized that use of a gene encoding an ADPglucose pyrophosphorylase enzyme which is only subject to modulators (activators/inhibitors) which are present in the selected plant at levels which do not significantly inhibit the catalytic activity will not require enzyme (gene) modification. These "unregulated" or "deregulated" ADPglucose pyrophosphorylase genes can then be inserted into plants as described herein to obtain transgenic plants having increased starch content.

For example, any ADPglucose pyrophosphorylase gene can be cloned into the *E. coli* B strain AC70R1-504 (Leung, 1986). This strain has a defective ADPglucose pyrophosphorylase gene, and is derepressed five- to seven-fold for the other glycogen biosynthetic enzymes. The ADPglucose pyrophosphorylase gene/ cDNA's can be put on a plasmid behind the *E. coli glgC* promoter or any other bacterial promoter. This construct can then be subjected to either site-directed or random mutagenesis. After mutagenesis, the cells would be plated on rich medium with 1% glucose. After the colonies have developed, the plates would be flooded with iodine solution (0.2 w/v% I₂, 0.4 w/v% KI in H₂O, Creuzet-Sigal, 1972). By comparison with an identical plate containing non-mutated *E. coli*, colonies that are producing more glycogen can be detected by their darker staining.

Since the mutagenesis procedure could have created promoter mutations, any putative ADPglucose pyrophosphorylase mutant from the first round screening will have to have the ADPglucose pyrophosphorylase gene recloned into non-mutated vector and the resulting plasmid will be screened in the same manner. The mutants that make it through both rounds of screening

will then have their ADPglucose pyrophosphorylase activities assayed with and without the activators and inhibitors. By comparing the mutated ADPglucose pyrophosphorylase's responses to activators and inhibitors to the non-mutated enzymes, the new mutant can be characterized.

5 The report by Plaxton and Preiss in 1987 demonstrates that the maize endosperm ADPglucose pyrophosphorylase has regulatory properties similar to those of the other plant ADPglucose pyrophosphorylases (Plaxton and Preiss 1987). They show that earlier reports claiming that the maize endosperm ADPglucose pyrophosphorylase had enhanced activity in the absence of
10 activator (3-PGA) and decreased sensitivity to the inhibitor (P_i), was due to proteolytic cleavage of the enzyme during the isolation procedure. By altering an ADPglucose pyrophosphorylase gene to produce an enzyme analogous to the proteolytically cleaved maize endosperm ADPglucose pyrophosphorylase, decreased allosteric regulation will be achieved.

15 To assay a liquid culture of *E. coli* for ADPglucose pyrophosphorylase activity, the cells are spun down in a centrifuge and resuspended in about 2 ml of extraction buffer (0.05 M glycylglycine pH 7.0, 5.0 mM DTE, 1.0 mM EDTA) per gram of cell paste. The cells are lysed by passing twice through a French Press. The cell extracts are spun in a microcentrifuge for 5 minutes, and the
20 supernatants are desalted by passing through a G-50 spin column.

 The enzyme assay for the synthesis of ADPglucose is a modification of a published procedure (Haugen et al., 1976). Each 100 μ l assay contains: 10 μ mole Hepes pH 7.7, 50 μ g BSA, 0.05 μ mole of [14 C]glucose-1-phosphate, 0.15 μ mole ATP, 0.5 μ mole $MgCl_2$, 0.1 μ g of crystalline yeast inorganic pyrophosphatase, 1 mM ammonium molybdate, enzyme, activators or inhibitors as desired,
25 and water. The reaction mixture is incubated at 37°C for 10 minutes, and is stopped by boiling for 60 seconds. The assay is spun down in a microcentrifuge, and 40 μ l of the supernatant is injected onto a Synchrom Synchropak AX-100 anion exchange HPLC column. The sample is eluted with 65 mM KPi pH 5.5.
30 Unreacted [14 C]glucose-1-phosphate elutes around 7-8 minutes, and [14 C]ADPglucose elutes at approximately 13 minutes. Enzyme activity is determined by the amount of radioactivity found in the ADPglucose peak.

 The plant ADPGPP enzyme activity is tightly regulated, by both positive (3-phosphoglycerate; 3-PGA) and negative effectors (inorganic phosphate; P_i)
35 (Ghosh and Preiss, 1966; Copeland and Preiss 1981; Sowokinos and Preiss

1982; Morell et al., 1987; Plaxton and Preiss, 1987; Preiss, 1988;) and the ratio of 3-PGA:P_i plays a prominent role in regulating starch biosynthesis by modulating the ADPGPP activity (Santarius and Heber, 1965; Heldt et al., 1977; Kaiser and Bassham, 1979). The plant ADPGPP enzymes are 5 heterotetramers of two large/"shrunk" and two small/"Brittle" subunits (Morell et al., 1987; Lin et al., 1988a, 1988b; Krishnan et al., 1986; Okita et al., 1990) and there is strong evidence to suggest that the heterotetramer is the most active form of ADPGPP. Support for this suggestion comes from the isolation of plant "starchless" mutants that are deficient in either of the 10 subunits (Tsai and Nelson, 1966; Dickinson and Preiss, 1969; Lin et al., 1988a, 1988b) and from the characterization of an "ADPGPP" homotetramer of small subunits that was found to have only low enzyme activity (Lin et al., 1988b). In addition, proposed effector interaction residues have been identified for both subunits (Morell et al., 1988). Direct evidence for the active form of the enzyme 15 and further support of the kinetic data reported for the purified potato enzyme comes from the expression of potato ADPGPP activity in *E. coli* and the comparison of the kinetic properties of this material and that from potato tubers (Iglesias et al., 1993).

Unregulated enzyme variants of the plant ADPGPP are identified and 20 characterized in a manner similar to that which resulted in the isolation of the *E. coli glgC16* and related mutants such as *glgC-SG5* and *CL1136*. A number of plant ADPGPP cDNA's, or portions of such cDNA's, for both the large and small subunits, have been cloned from both monocots and dicots (Anderson et al., 1989a; Olive et al., 1989; Muller et al., 1990; Bhave et al., 1990; du Jardin 25 and Berhin, 1991; Smith-White and Preiss, 1992). The proteins encoded by the plant cDNA's, as well as those described from bacteria, show a high degree of conservation (Bhave et al., 1990). In particular, a highly conserved region, also containing some of the residues implicated in enzyme function and effector interactions, has been identified (Morell et al., 1988; du Jardin and Berhin, 30 1991). Clones of the potato tuber ADPGPP subunit genes have been isolated. These include a complete small subunit gene, assembled by addition of sequences from the first exon of the genomic clone with a nearly full-length cDNA clone of the same gene, and an almost complete gene for the large subunit. The nucleotide sequence (SEQ ID NO:7) and the amino acid sequence 35 (SEQ ID NO:8) of the assembled small subunit gene is given below. The

-12-

nucleotide sequence presented here differs from the gene originally isolated in the following ways: a *Bgl*III+*Nco*I site was introduced at the ATG codon to facilitate the cloning of the gene into *E. coli* and plant expression vectors by site directed mutagenesis utilizing the oligonucleotide primer sequence

5 GTTGATAACAAGATCTGTTAACCATGGCGGCTTCC (SEQ ID NO:11).

A *Sac*I site was introduced at the stop codon utilizing the oligonucleotide primer sequence

CCAGTTAAAACGGAGCTCATCAGATGATGATTC (SEQ ID NO:12).

The *Sac*I site serves as a 3' cloning site. An internal *Bgl*III site was removed
10 utilizing the oligonucleotide primer sequence

GTGTGAGAACATAAATCTTGGATATGTTAC (SEQ ID NO:13).

This assembled gene was expressed in *E. coli* under the control of the *recA* promoter in a *P recA-gene10L* expression cassette (Wong et al., 1988) to produce measurable levels of the protein. An initiating methionine codon is
15 placed by site-directed mutagenesis utilizing the oligonucleotide primer sequence

GAATTCACAGGGCCATGGCTCTAGACCC (SEQ ID NO:14)

to express the mature gene.

The nucleotide sequence (SEQ ID NO:9) and the amino acid sequence
20 (SEQ ID NO:10) of the almost complete large subunit gene is given below. An initiating methionine codon has been placed at the mature N-terminus by site-directed mutagenesis utilizing the oligonucleotide primer sequence

AAGATCAAACCTGCCATGGCTTACTCTGTGATCACTACTG (SEQ ID NO:15). The purpose of the initiating methionine is to facilitate the expression

25 of this large subunit gene in *E. coli*. A *Hind*III site is located 103 bp after the stop codon and serves as the 3' cloning site. The complete large ADPGPP gene is isolated by the 5' RACE procedure (Rapid Amplification of cDNA Ends; Frohman, 1990; Frohman et al., 1988; Loh et al., 1989). The oligonucleotide primers for this procedure are as follows:

30 1) GGGAATTCAAGCTTGGATCCCGGGCCCCCCCCCCCCCCCCC
(SEQ ID NO:16);

2) GGGAATTCAAGCTTGGATCCCGGG (SEQ ID NO:17); and

3) CCTCTAGACAGTCGATCAGGAGCAGATGTACG (SEQ ID NO:18).

The first two are the equivalent to the ANpolyC and the AN primers of Loh et
35 al. (1989), respectively, and the third is the reverse complement to a sequence

-13-

in the large ADPGPP gene. The PCR 5' sequence products are cloned as *EcoRI/HindIII/BamHI-PstI* fragments and are easily assembled with the existing gene portion.

The weakly regulated enzyme mutants of ADPGPP are identified by initially scoring colonies from a mutagenized *E. coli* culture that show elevated glycogen synthesis, by iodine staining of 24-48 hour colonies on Luria-Agar plates containing glucose at 1%, and then by characterizing the responses of the ADPGPP enzymes from these isolates to the positive and negative effectors of this activity (Cattaneo et al., 1969; Preiss et al., 1971). A similar approach is applied to the isolation of such variants of the plant ADPGPP enzymes. Given an expression system for each of the subunit genes, mutagenesis of each gene is carried out separately, by any of a variety of known means, both chemical or physical (Miller, 1972) on cultures containing the gene or on purified DNA. Another approach is to use a PCR procedure (Ehrlich, 1989) on the complete gene in the presence of inhibiting Mn⁺⁺ ions, a condition that leads to a high rate of misincorporation of nucleotides. A PCR procedure may also be used with primers adjacent to just a specific region of the gene, and this mutagenized fragment then recloned into the non-mutagenized gene segments. A random synthetic oligonucleotide procedure may also be used to generate a highly mutagenized short region of the gene by mixing of nucleotides in the synthesis reaction to result in misincorporation at all positions in this region. This small region is flanked by restriction sites that are used to reinsert this region into the remainder of the gene. The resultant cultures or transformants are screened by the standard iodine method for those exhibiting glycogen levels higher than controls. Preferably this screening is carried out in an *E. coli* strain deficient only in ADPGPP activity and is phenotypically glycogen-minus and that is complemented to glycogen-plus by *glgC*. The *E. coli* strain should retain those other activities required for glycogen production. Both genes are expressed together in the same *E. coli* host by placing the genes on compatible plasmids with different selectable marker genes, and these plasmids also have similar copy numbers in the bacterial host to maximize heterotetramer formation. An example of such an expression system is the combination of pMON17335 and pMON17336 (Iglesias et al., 1993). The use of separate plasmids enables the screening of a mutagenized population of one gene alone, or in conjunction with the second gene following transformation into

-14-

a competent host expressing the other gene, and the screening of two mutagenized populations following the combining of these in the same host. Following re-isolation of the plasmid DNA from colonies with increased iodine staining, the ADPGPP coding sequences are recloned into expression vectors, 5 the phenotype verified, and the ADPGPP activity and its response to the effector molecules determined. Improved variants will display increased V_{\max} , reduced inhibition by the negative effector (P_i), or reduced dependence upon activator (3-PGA) for maximal activity. The assay for such improved characteristics involves the determination of ADPGPP activity in the presence of P_i at 10 0.045 mM ($I_{0.5} = 0.045$ mM) or in the presence of 3-PGA at 0.075 mM ($A_{0.5} = 0.075$ mM). The useful variants will display <40% inhibition at this concentration of P_i or display >50% activity at this concentration of 3-PGA. Following the isolation of improved variants and the determination of the subunit or subunits responsible, the mutation(s) are determined by nucleotide sequencing. 15 The mutation is confirmed by recreating this change by site-directed mutagenesis and reassay of ADPGPP activity in the presence of activator and inhibitor. This mutation is then transferred to the equivalent complete ADPGPP cDNA gene, by recloning the region containing the change from the altered bacterial expression form to the plant form containing the amyloplast 20 targeting sequence, or by site-directed mutagenesis of the complete native ADPGPP plant gene.

Example 1

Construction of DNA Vectors for *glgC16* Expression

25 To express the *E. coli glgC16* gene in plant cells, and to target the enzyme to the plastids, the gene needed to be fused to a DNA encoding the plastid-targeting transit peptide (hereinafter referred to as the CTP/ADP-glucose pyrophosphorylase gene), and to the proper plant regulatory regions. This was accomplished by cloning the *glgC16* gene into a series of plasmid 30 vectors that contained the needed sequences.

The plasmid pLP226 contains the *glgC16* gene on a *HincII* fragment, cloned into a pUC8 vector at the *HincII* site (Leung et al. 1986). pLP226 was obtained from Dr. Jack Preiss at Michigan State University, and was transformed into frozen competent *E. coli* JM101 cells, prepared by the 35 calcium chloride method (Sambrook et al., 1989). The transformed cells were

-15-

plated on 2XYT (infra) plates that contained ampicillin at 100 µg/ml. The plasmid pLP226 was purified by the rapid alkaline extraction procedure (RAE) from a 5 ml overnight culture (Birnboim and Doly, 1979).

To fuse the *glgC16* gene to the DNA encoding the chloroplast transit peptide, a NcoI site was needed at the 5' end of the gene. A SacI site downstream of the termination codon was also needed to move the CTP/ADP-glucose pyrophosphorylase gene into the next vector. In order to introduce these sites, a PCR reaction (#13) was run using approximately 20 ng of rapid alkaline extraction-purified plasmid pLP226 for a template. The reaction was set up following the recommendations of the manufacturer (Perkin Elmer Cetus). The primers were QSP3 and QSP7. QSP3 was designed to introduce the NcoI site that would include the start codon for the *glgC16* gene. The QSP7 primer hybridized in the 3' nontranslated region of the *glgC16* gene and added a SacI site. The Thermal Cycler was programmed for 30 cycles with a 15 min 94°C denaturation step, a 2 min 50°C annealing step, and a 3 min 72°C extension step. After each cycle, the extension step was increased by 15 sec. QSP3 Primer: 5' GGAGTTAGCCATGGTTAGTTTAGAG 3' (SEQ ID NO: 19) QSP7 Primer:

5' GGCCGAGCTCGTCAACGCCGCTCTGCGATTTGTGC 3' (SEQ ID NO: 20)

The PCR product was cloned into vector pGEM3zf+ (Promega, Madison, WI), which had been digested with SacI and Hind III and had the DNA for the modified *Arabidopsis* small subunit CTP ligated at the HindIII site. The DNA and amino acid sequences of this CTP are shown in SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

The linearized vector was treated with 5 units of calf intestinal alkaline phosphatase for 30 min at 56°C. Then, both the vector and the PCR #13 fragment, which had the *glgC16* gene with the new NcoI and SacI sites, were run on an agarose gel and the fragments were purified by binding to DEAE membranes. The protocol used for the fragment purification with the DEAE membrane is from Schleicher and Schuell, and is titled "Binding and Recovery of DNA and RNA Using S and S DEAE Membrane."

Ligation #5 fused the *glgC16* gene to the DNA for the modified *Arabidopsis* SSU CTP with the pGEM3zf+. The ligation contained 3 µl of vector that had been digested with NcoI and SacI, along with 3 µl of the PCR #13 product, that had also been cut with NcoI and SacI and repurified on a gel.

-16-

5 μ l (of 20 μ l total) of ligation #5 was transformed into frozen competent JM101 cells, and the transformed cells were plated on 2XYT plates (16 g/l Bacto-tryptone, 10 g/l yeast extract, 10 g/l NaCl, pH 7.3, and solidified with 1.5% agar) containing ampicillin.

5 Sample 1 was picked from a plate after overnight growth. This sample was inoculated into 4 ml of 2XYT media and grown overnight at 37 °C. The plasmid was isolated by the rapid alkaline extraction procedure, and the DNA was digested with EcoRI, NcoI, and EcoRI and NcoI together. The digest was separated on an agarose gel, and the expected fragments were observed. The
10 plasmid isolated from sample 1 of was designated pMON20100, and consisted of pGEM3zf+, the DNA for the modified *Arabidopsis* SSU CTP, and the *glgC16* gene. The fusion was in the orientation that allowed it to be transcribed from the SP6 polymerase promoter.

To test this construct for import of the ADPglucose pyrophosphorylase
15 into isolated lettuce chloroplasts, the CTP/ADPglucose pyrophosphorylase fusion needed to be transcribed and translated to produce [³⁵S]-labeled ADPglucose pyrophosphorylase. To make a DNA template for transcription by the SP6 polymerase, the CTP/ADPglucose pyrophosphorylase region of pMON20100 was amplified by PCR to generate a large amount of linear DNA.
20 To do this, about 0.1 μ l of pMON20100, that had been purified by rapid alkaline extraction, was used as a template in PCR reaction #80. The primers were a commercially available SP6 promoter primer (Promega) and the oligo QSP7 (SEQ ID NO:20). The SP6 primer hybridized to the SP6 promoter in the vector, and included the entire SP6 promoter sequence. Therefore, a PCR product
25 primed with this oligo will contain the recognition sequence for the SP6 polymerase. The QSP7 (SEQ ID NO:20) primer will hybridize in the 3' nontranslated region of the *glgC16* gene. This is the same primer that was used to introduce a SacI site downstream of the *glgC16* termination codon. The Thermal Cycler was programmed for 30 cycles with a 1 min denaturation at
30 94°C, a 2 min annealing at 55°C, and a 3 min extension at 72°C. After each cycle, 15 sec were added to the extension step.

SP6 Promoter Primer: 5' GATTAGGTGACACTATAG 3' (SEQ ID NO:21)

5 μ l of PCR reaction #80 was run on an agarose gel and purified by binding to DEAE membrane. The DNA was eluted and dissolved in 20 μ l of TE.
35 2 μ l of the gel-purified PCR #80 product was used in an SP6 RNA polymerase in

vitro transcription reaction. The reaction conditions were those described by the supplier (Promega) for the synthesis of large amounts of RNA (100 µl reaction). The RNA produced from the PCR reaction #80 DNA was used for *in vitro* translation with the rabbit reticulocyte lysate system (Promega).

5 ³⁵S-labeled protein made from pMON20100 (i.e., PCR reaction# 80) was used for an *in vitro* chloroplast import assay as previously described. After processing the samples from the chloroplast import assay, the samples were subjected to electrophoresis on SDS-PAGE gels with a 3-17% polyacrylamide gradient. The gel was fixed for 20-30 min in a solution with 40% methanol and
10 10% acetic acid. Then, the gel was soaked in EN³HANCE™ for 20-30 min, followed by drying the gel on a gel dryer. The gel was imaged by autoradiography, using an intensifying screen and an overnight exposure. The results demonstrated that the fusion protein was imported into the isolated chloroplasts.

15 The construct in pMON20100 next was engineered to be fused to the enhanced CaMV 35S promoter (Kay, R. 1987) and the NOS 3' end (Bevan, M. 1983) isolated from pMON999. PCR reaction 114 contained plasmid pMON20100 as a template, and used primers QSM11 and QSM10. QSM11 annealed to the DNA for the modified *Arabidopsis* SSU CTP and created a
20 BglII site 7 bp upstream from the ATG start codon. QSM10 annealed to the 3' end of the *glgC16* gene and added an XbaI site immediately after the termination codon, and added a SacI site 5 bp after the termination codon. The SacI site that had earlier been added to the *glgC16* gene was approximately 100 bp downstream of the termination codon. The Thermal Cycler was
25 programmed for 25 cycles with a 1 min 94°C denaturation, a 2 min 55°C annealing, and a 3 min 72°C extension step. With each cycle, 15 sec was added to the extension step.

QSM11 Primer (SEQ ID NO:22):

5' AGAGAGATCTAGAACAATGGCTTCCTCTATGCTCTCTTCCGC 3'

30 QSM10 Primer (SEQ ID NO:23):

5' GGCCGAGCTCTAGATTATCGCTCCTGTTTATGCCCTAAC 3'

95µl (from 100 µl total volume) of PCR reaction #114 was ethanol precipitated, and resuspended in 20 µl of TE. 5 µl of this was digested with BglII (4 units) and SacI (10 units) overnight at 37°C. 5 µl (5 µg) of the vector,
35 pMON999, which contains the enhanced CaMV 35S promoter and the NOS

3' end, was digested in the same manner. After digestion with the restriction enzymes, the DNAs were run on an agarose gel and purified by binding to DEAE membranes. Each of the DNAs were dissolved in 20 μ l of TE. 1 μ l of PCR 114 was ligated with 3 μ l of the vector, in a total volume of 20 μ l. The 5 ligation mixture was incubated at 14°C for 7 hr. 10 μ l of the ligation was transformed into frozen competent MM294 cells and plated on LB plates (10 g/l Bacto-tryptone, 5 g/l yeast extract, 10 g/l NaCl, and 1.5% agar to solidify) with 100 μ g/ml ampicillin. Colonies were picked and inoculated into tubes with 5 ml of LB media with 100 μ g/ml ampicillin, for overnight growth. The 5 ml overnight 10 cultures were used for rapid alkaline extractions to isolate the plasmid DNAs. The DNAs were digested with EcoRI, and separate aliquots were digested with NotI. After analyzing these samples on agarose gels, the plasmid pMON20102 was confirmed to have the 497 bp EcoRI fragment that is characteristic of the *glgC16* gene. This plasmid also contained the 2.5 kb NotI fragment which 15 contained the enhanced CaMV 35S promoter, the DNA for the modified *Arabidopsis* SSU CTP, the *glgC16* gene, and the NOS 3' end.

The pMON20102 plasmid was then used to construct a DNA vector which would express the *glgC16* gene in a tuber-specific manner and would be used for the transformation of potato. This construct causes specific expres- 20 sion of the ADPGPP in potato tubers and increases the level of starch in the tubers.

The vector used in the potato transformation is a derivative of the *Agrobacterium* mediated plant transformation vector pMON886. The pMON886 plasmid is made up of the following well characterized segments of 25 DNA. A 0.93 kb fragment isolated from transposon Tn7 which encodes bacterial spectinomycin/streptomycin (Spc/Str) resistance and is a determinant for selection in *E. coli* and *Agrobacterium tumefaciens* (Fling et al., 1985). This is joined to a chimeric kanamycin resistance gene engineered for plant expression to allow selection of the transformed tissue. The chimeric gene 30 consists of the 0.35 kb cauliflower mosaic virus 35S promoter (P-35S) (Odell et al., 1985), the 0.83 kb neomycin phosphotransferase type II gene (NPTII), and the 0.26 kb 3'-nontranslated region of the nopaline synthase gene (NOS 3') (Fraley et al., 1983). The next segment is a 0.75 kb origin of replication from the RK2 plasmid (ori-V) (Stalker et al., 1981). It is joined to a 3.1 kb *SalI* to 35 *PvuI* segment of pBR322 which provides the origin of replication for

maintenance in *E. coli* (ori-322) and the *bom* site for the conjugational transfer into the *Agrobacterium tumefaciens* cells. Next is a 0.36 kb *PvuI* fragment from the pTiT37 plasmid which contains the nopaline-type T-DNA right border region (Fraley et al., 1985).

5 The *glgC16* gene was engineered for expression primarily in the tuber by placing the gene under the control of a tuber-specific promoter. The GlgC16 protein was directed to the plastids within the plant cell due to its synthesis as a C-terminal fusion with a N-terminal protein portion encoding a chloroplast targeting sequence (CTP) derived from that from the SSU 1A gene from
10 *Arabidopsis thaliana* (Timko et al., 1989). The CTP portion is removed during the import process to liberate the GlgC16 enzyme. Other plant expression signals also include the 3' polyadenylation sequences which are provided by the NOS 3' sequences located downstream from the coding portion of the expression cassette. This cassette was assembled as follows: The patatin promoter was
15 excised from the pBI241.3 plasmid as a *HindIII*-*BamHI* fragment (The pBI241.3 plasmid contains the patatin-1 promoter segment comprising from the *AccI* site at 1323 to the *DraI* site at 2289 [positions refer to the sequence in Bevan et al., 1986] with a *HindIII* linker added at the former and a *BamHI* linker added at the latter position; Bevan et al., 1986) and ligated together with
20 the CTP1-*glgC16* fusion (the *BglII*-*SacI* fragment from pMON20102) and pUC-type plasmid vector cut with *HindIII* and *SacI* (these cloning sites in the vector are flanked by *NotI* recognition sites). The cassette was then introduced, as a *NotI* site in pMON886, such that the expression of the *glgC16* gene is in the same orientation as that of the NPTII (kanamycin) gene. This derivative
25 is named pMON20113, illustrated in Figure 7 of Kishore, WO 91/19806.

Plant Transformation/Regeneration

The pMON20113 vector was mobilized into disarmed *Agrobacterium tumefaciens* strain by the triparental conjugation system using the helper
30 plasmid pRK2013 (Ditta et al., 1980). The disarmed strain ABI was used, carrying a Ti plasmid which was disarmed by removing the phytohormone genes responsible for crown gall disease. The ABI strain is the A208 *Agrobacterium tumefaciens* carrying the disarmed pTiC58 plasmid pMP90RK (Koncz and Schell, 1986). The disarmed Ti plasmid provides the *trfA* gene
35 functions required for autonomous replication of the pMON vector after the

conjugation into the ABI strain. When the plant tissue is incubated with the ABI::pMON conjugate, the vector is transferred to the plant cells by the vir functions encoded by the disarmed pMP90RK Ti plasmid.

The pMON20113 construct, encoding the bacterial ADPGPP gene (SEQ 5 ID NO:1), was transformed into the Russet Burbank potato variety Williams by the following procedure. To transform Russet Burbank potatoes, sterile shoot cultures of Russet Burbank are maintained in sundae cups containing 8 ml of PM medium supplemented with 25 mg/L ascorbic acid (Murashige and Skoog (MS) inorganic salts, 30 g/l sucrose, 0.17 g/l $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$, 0.4 mg/l 10 thiamine-HCl, and 100 mg/l myo-inositol, solidified with 2 g/l Gelrite at pH 6.0). When shoots reach approximately 5 cm in length, stem internode segments of 3-5 mm are excised and inoculated with a 1:10 dilution of an overnight culture of *Agrobacterium tumefaciens* from a 4 day old plate culture. The stem explants are co-cultured for 2 days at 20°C on a sterile filter paper placed over 1.5 ml of 15 a tobacco cell feeder layer overlaid on 1/10 P medium (1/10 strength MS inorganic salts and organic addenda without casein as in Jarret et al. (1980), 30 g/l sucrose and 8.0 g/l agar). Following co-culture, the explants are transferred to full strength P-1 medium for callus induction, composed of MS inorganic salts, organic additions as in Jarret et al. (1980), with the exception of casein, 20 5.0 mg/l zeatin riboside (ZR), and 0.10 mg/l naphthalene acetic acid NAA (Jarret et al., 1980a, 1980b). Carbenicillin (500 mg/l) and cefotaxime (100 mg/L) are included to inhibit bacterial growth, and 100 mg/l kanamycin is added to select for transformed cells.

After 4 weeks, the explants are transferred to medium of the same 25 composition, but with 0.3 mg/l gibberellic acid (GA3) replacing the NAA (Jarret et al., 1981) to promote shoot formation. Shoots begin to develop approximately 2 weeks after transfer to shoot induction medium. These shoots are excised and transferred to vials of PM medium for rooting. After about 4 weeks on the rooting medium, the plants are transferred to soil and are gradually hardened 30 off. Shoots are tested for kanamycin resistance conferred by the enzyme neomycin phosphotransferase II, by placing the shoots on PM medium for rooting, which contains 50 mg/L kanamycin, to select for transformed cells.

Russet Burbank Williams plants regenerated in culture were transplanted into 6 inch (~15.24 cm) pots and were grown to maturity under 35 greenhouse conditions. Tubers were harvested and were allowed to suberize at

room temperature for two days. All tubers greater than 2 cm. in length were collected and stored at 3°C under high humidity.

Specific Gravity and Starch Determinations of Stored Tubers

5 Specific gravity (SG) was determined after 3 and 4 months of cold (3 °C) storage for the largest 2 or 3 tubers from each plant, with typical weights being 20-40 grams per tuber. Tubers were allowed to warm to room temperature for a few hours prior to specific gravity determination, but were not allowed to
10 air/weight in water method, where $SG = \text{weight in air} / (\text{weight in air} - \text{weight in water})$. Calculations for percent starch and percent dry matter based on SG were according to the following formulas (von Scheele, 1937):

$$\% \text{ starch} = 17.546 + (199.07)(SG - 1.0988)$$

$$\% \text{ dry matter} = 24.182 + (211.04)(SG - 1.0988)$$

15 Starch analysis was performed on fresh, center sections of stored tuber tissue as described (Lin et al., 1988). Tubers were not allowed to warm before harvesting tissue. Briefly, approximately 100 mg. center sections were cut, weighed, placed in 1.5 ml centrifuge tubes, and frozen on dry ice. The tissue was then dried to a stable weight in a Savant Speed-Vac Concentrator, and final dry
20 weight was determined. Soluble sugars were first removed by extracting three times with 1 ml. of 80% ethanol at 70 °C, for 20 minutes per treatment. After the final incubation, all remaining ethanol was removed by desiccation in a Speed- Vac Concentrator. The solid material was resuspended in 400 µl 0.2 M potassium hydroxide, ground, and then incubated for 30 min. at 100 °C to
25 solubilize the starch. The solutions were cooled and neutralized by addition of 80 µl 1N acetic acid. Starch was degraded to glucose by treatment with 14.8 units of pancreatic alpha-amylase (Sigma Chemical, St. Louis) for 30 min. at 37 °C, followed by 10 units of amyloglucosidase (Sigma Chemical, St. Louis) for 60 min. at 55 °C. Glucose released by the enzymatic digestions was measured
30 using the Sigma (St. Louis) hexokinase kit, and these values were used to calculate starch content.

Sugar Analysis

Tubers were stored at 3 °C and were not allowed to recondition at room
35 temperature prior to sugar analysis. Center cuts from stored tubers were

-22-

obtained, fresh weights determined, and the tissue was frozen on dry ice prior to desiccation in Savant Speed-Vac Concentrator. Approximate fresh weight per sample was 100 mg. Dry tuber material was coarsely ground, and sugars were extracted three times with 0.5 ml 80% ethanol at 70 °C for 20 minutes per 5 extraction. After each incubation, the insoluble material was spun down for 2 minutes in a microcentrifuge and the supernatant collected. The supernatants from all three extractions were combined, dried down, and resuspended in 1 ml 100 mM Tris buffer, pH 7.5. For each sugar analysis, 10µl of sample was used.

For each sample, glucose content was determined using a Glucose [HK] 10 diagnostic kit (Sigma Chemical Co., St. Louis, MO) according to manufacturers protocol. Briefly, 1 mL of reconstituted reagent was incubated with 10 µl of sample at room temperature for 10 minutes, and the sample concentration determined by measuring absorbance at 340 nm subtracting the absorbance of 10 µl of sample in water. Percent glucose was then calculated by the equation:

15 % glucose = $[(A_{340} \times 2.929) / \text{mg. fresh weight}] \times 100\%$

Fructose content was determined by adding 1µg of phosphoglucosomerase to the above reaction for glucose determination, and subtracting the resultant percent glucose + fructose value from percent glucose. Sucrose content was determined by addition of 1 µg phosphoglucosomerase and 100µg yeast 20 invertase to the glucose HK assay, and extending incubation time to 30 minutes at room temperature. Percent sucrose was determined as above, subtracting the values obtained for glucose and fructose content.

Western Blot Analysis of Stored Tubers

25 Tubers stored at 3 °C were not allowed to warm prior to isolation of tissue for analysis. For Western blot analysis, proteins were extracted from desiccated, coarsely powdered tuber tissue by grinding 1:1 in 100 mM Tris pH 7.5, 35 mM KCl, 5 mM dithiothreitol, 5 mM ascorbate, 1 mM EDTA, 1 mM benzamidine, and 20% glycerol. The protein concentration of the extract was 30 determined using the Pierce BCA method, and proteins were separated on 3-17% SDS polyacrylamide gels (Laemmli, 1970). E. coli ADPGPP was detected using goat antibodies raised against purified E. coli ADPGPP and alkaline phosphatase conjugated rabbit anti-goat antibodies (Promega, Madison, WI).

Fry Color Determination

Eight transgenic potato lines expressing the *E. coli glgC16* gene, and 20 control lines consisting of a combination of lines from the pMON20113 transformation event which do not express the *E. coli glgC16* gene, and several 5 nontransgenic Russet Burbank control lines, were grown under field conditions in Parma, Idaho. Tubers were harvested and stored for two months at 40 °C. Fry color was determined for all potato lines by taking center cuts from representative samples from each line and frying at 375 °F in soybean oil for 3 minutes and 30 seconds. Fry color was determined by photovoltaic measure- 10 ment and values were reported according to the USDA color class chart for frozen french fries.

Results

All tubers were harvested from plants of the same variety (Russet 15 Burbank Williams 82), the same age, and grown side by side under identical growth conditions. Western blot analysis showed that levels of *E. coli* ADPGPP were essentially equivalent to levels determined at harvest (Table 1), suggesting that the levels of *E. coli* ADPGPP protein are stable during cold storage. Analysis of tubers stored at 3 °C under high humidity shows that those 20 expressing the *E. coli glgC16* gene accumulate 5 - to 6-fold less reducing sugar than do control tubers (Tables 2, 3, 4, 5, and 6). Sucrose levels were comparable between control and transgenic tubers, while starch levels were significantly higher in the transgenic tubers. These results suggest that as starch is degraded during storage, the sugars formed tend to be resynthesized into starch 25 in those tubers expressing the *E. coli glgC16* gene, while in control tubers the sugars tend to accumulate.

Transgenic potato plants expressing the *E. coli glgC16* gene have been grown under field conditions and tubers from GlgC16 potato lines were stored at 40 °F (4 °C) along with tubers from several different control lines. Fry color, 30 which directly correlated with sugar content, was determined after two months cold storage. The average fry color in the transgenic potato tubers was significantly improved (lighter) relative to that in control tubers (darker color) stored under identical conditions (Table 7), demonstrating that sugar levels were lower in the tubers expressing the *E. coli glgC16* gene. Direct measurement of 35 reducing sugar content in a sample of the field grown tubers stored for 14 weeks

at 3 °C supports the fry color results in that tubers expressing the *E. coli glgC16* gene contained significantly less reducing sugar than controls (Table 8). Tubers from transgenic potato plants were tested for rate and degree of reconditioning following cold storage. The fry color of transgenic lines which produce 5 tubers having a specific gravity greater than 1.083 indicated an increased rate and degree of reconditioning at 65 °F as compared to controls (Table 9).

TABLE 1

Expression of *E. coli* ADPGPP in potato tubers at harvest and after 3 months cold storage. *E. coli* ADPGPP levels were estimated from Western blot analysis by comparison to known standards. Values are given in ng GlgC16 per 50µg extracted tuber protein.

<u>Line</u>	<u>ng GlgC16</u>	
	<u>Harvest</u>	<u>3 Months</u>
15 353c	20-25	20-25
535c	25-30	20-25
448a	25-30	25-30
182a	2	0.5-1
199a	20-25	20-25
20 288c	20-25	20-25
194a	15-20	15-20
524a	10	15-20

TABLE 2

Sugar and starch content (Dry Weight measurements) in 3 month cold stored 25 tubers. Reducing sugars are glucose and fructose, and total sugars are reducing sugars plus sucrose. Values (percent dry weight) represent the averages from 9 *glgC16* + high starch potato lines, and 11 control (*glgC16*-) potato lines stored for 3 months at 3 °C.

	<u>Reducing Sugars</u>	<u>Sucrose</u>	<u>Total Sugars</u>	<u>Starch</u>
30 <i>glgC16</i> +	1.5	1.2	2.6	59.5
Control	7.0	0.8	7.8	53.7

TABLE 3

Sugar and starch content (Fresh Weight measurements) in 4 month cold stored 35 tubers. Reducing sugars are glucose and fructose, and total sugars are reducing

-25-

sugars plus sucrose. Values (percent fresh weight) represent the averages from 9 *glgC16* + high starch potato lines, and 11 control (*glgC16*-) potato lines stored for 4 months at 3 °C.

	<u>Reducing Sugars</u>	<u>Sucrose</u>	<u>Total Sugars</u>	<u>Starch</u>
5 <i>glgC16</i> +	0.1	0.1	0.3	9.9
Control	0.8	0.2	1.0	6.0

TABLE 4

Reducing sugar content of potato tubers after 4 months cold storage. Numbers 10 of plant lines containing sugar levels within the ranges shown are reported.

Percentages are based on fresh weight.

	<u>Percent Reducing Sugars</u>					
	<u>0-.2</u>	<u>.2-.4</u>	<u>.4-.6</u>	<u>.6-.8</u>	<u>.8-1.0</u>	<u>1.0+</u>
Control lines	0	2	0	4	2	3
15 <i>glgC16</i> lines	6	3	0	0	0	0

TABLE 5

Total sugar content of potato tubers after 4 months cold storage. Numbers of plant lines containing sugar levels within the ranges shown are reported.

20 Percentages are based on fresh weight.

	<u>Percent Total Sugars</u>					
	<u>0-1</u>	<u>1-2</u>	<u>2-3</u>	<u>3-4</u>	<u>4-5</u>	<u>5+</u>
Control lines	0	0	0	2	3	6
<i>glgC16</i> lines	3	4	2	0	0	0

25

TABLE 6

Starch content of potato tubers after 4 months cold storage. Numbers of plant lines containing starch levels within the ranges shown are reported.

Percentages are based on fresh weight.

	<u>Percent Starch</u>					
	<u>2-4</u>	<u>4-6</u>	<u>6-8</u>	<u>8-10</u>	<u>10-12</u>	<u>12-14</u>
Control lines	1	6	3	1	0	0
<i>glgC16</i> lines	0	0	2	3	3	1

-26-

TABLE 7

Average fry color of field grown tubers after 2 months cold storage at 40 °F. The fry color rating was assigned according to the USDA published color standards for frozen fried potatoes. In this rating, 0 = very light color and 4 = 5 very dark color. Numbers of plant lines having fry colors within the ranges shown are reported.

	<u>Fry Color Rating</u>			
	<u>2-2.49</u>	<u>2.5-2.99</u>	<u>3.0-3.49</u>	<u>3.5-4.0</u>
Control lines	0	0	4	16
10 <i>glgC16</i> lines	1	1	6	0

TABLE 8

Reducing sugar content of field grown potato tubers after 14 weeks storage at 3 °C. Numbers of plant lines containing sugar levels within the ranges shown are 15 reported. Percentages are based on fresh weight.

	<u>Percent Reducing Sugars</u>			
	<u>0.5-1</u>	<u>1-1.5</u>	<u>1.5-2</u>	<u>2-2.5</u>
Control lines	0	4	2	2
<i>glgC16</i> lines	4	2	2	0

20

EXAMPLE 2

Following storage at low temperatures, potatoes are frequently unacceptable for frying due to elevated sugar levels. These stored potatoes are improved by treatments such as blanching or reconditioning; the former treatment removes sugars by treatment of the potato slices with hot water and in the latter the sugars are metabolized during storage of the tubers at higher temperatures (~ 65 °F). Blanching is used to inactivate enzymes primarily but when sugars are high the times employed in this step are extended to many times the normal. The extension of this step results in lower recovery of product, a loss of flavor, is time consuming, requires high energy input, and produces waste material with high biological oxygen demand and thus poses additional limitations on the disposal of the waste water. Reconditioning requires additional controlled temperature storage facilities and optimal results may require a number of steps at different temperatures. Sprouting and incidence of disease will increase at the higher temperatures and

-27-

with time. The fry color of fries made from cold-stored GlgC16 tubers were frequently lower (better) than controls and in some cases were low enough even after 3-4 months that no reconditioning would be required. These tests have been extended to tubers stored for 2 months at 50 °F and then for 3 months at 5 38 °F and include a measure of the rate of reconditioning also.

Tubers from plants transformed with the following vectors were tested: pMON17316 (with the patatin 3.5 promoter) and pMON17279 (with the small subunit of potato ADPGPP); as well as tubers from plants containing the patatin 1.0 promoter /*glgC16* vector described above. These vectors were
10 constructed as follows:

The patatin 3.5 promoter was obtained from the plasmid pPBI240.7 (Bevan, 1986). The majority of the 3.5 promoter was excised from pPBI240.7, from the *Hind*III site (-3500) to the *Xba*I site at -337, and combined with the remainder of the promoter, from the *Xba*I site to a *Bgl*II site at +22 (formerly a
15 *Dra*I site), in a triple ligation into a vector which provided a *Bgl*II site to form pMON17280. This latter plasmid then served as the vector for the triple ligation of the complete 3.5 promoter and the plastid target peptide-*GlgC16* fusion from pMON20102, described above to form the tuber expression cassette (in pMON17282). This cassette, consisting of the patatin 3.5 promoter,
20 the plastid target peptide-*GlgC16* fusion, and the NOS 3' sequences, was introduced into the plant transformation vector pMON17227, a Ti plasmid vector disclosed and described by Barry et al. in WO 92/04449 (1991), incorporated herein by reference, on a *Not*I fragment to form pMON17316. See Figure 1.

The promoter for the potato tuber ADPGPP small subunit gene,
25 SEQ ID NO:24, was obtained as a *Xba*I-*Bgl*II fragment of the genomic clone 1-2 and inserted into the *Xba*I and *Bam*HI site of Bluescript II KS- (Nakata et al., 1992). The promoter fragment used consists of the portion from the *Cl*aI site about 2.0 kb 5' from the putative initiation methionine and extending to the *Hind*III site located 12 bp before this ATG. A *Bgl*II site was placed adjacent to
30 this *Hind*III site by subcloning through another pUC vector, and was linked through this latter site to the fusion of the CTP targeting and the *glgC16* coding sequences. This cassette, with a plant 3' recognition sequence was cloned into plant transformation vectors to form pMON17279 (also includes a cassette in which the *E. coli uidA* [GUS] gene is expressed from the same small potato
35 ADPGPP promoter). See Figure 2.

-28-

These vectors were inserted into potato cells by *Agrobacterium* transformation followed by glyphosate selection. To transform potatoes using glyphosate as a selectable agent, the appropriate *Agrobacterium* was grown overnight in 2 ml of LB SCK. The following day, the bacteria was diluted 1:10 with MSO or until an optical density reading of 0.2-0.33 was established. Leaves from the stems of potato plants that had been grown under sterile conditions for three weeks on PM media supplemented with 25 mg/ml ascorbic acid were removed, stems were cut into 3-5 mm segments and inoculated with diluted bacteria as described previously. Explants were placed onto prepared co-culture plates. The co-culture plates contained 1/10 MSO with 1.5 mL of TxD cells overlain with wetted filter paper. About 50 explants were placed per plate. After 2 days co-culture period, explants were placed onto callus induction media which contains 5.0 mg/l Zeatin Riboside, 10 mg/l AgNO₃ and 0.1 mg/l NAA for 2 days. Explants were subsequently transferred onto callus induction media which contained 0.025 mM glyphosate for selection. After 4 weeks, explants were placed onto shoot induction media which contained 5.0 mg/l Zeatin Riboside + 10 mg/l AgNO₃ and 0.3 mg/l GA₃, with 0.025 mM glyphosate for selection. Shoots began to appear at 8 weeks. Explants were transferred to fresh shoot induction media every 4 weeks for 12 weeks. Shoots were excised and placed on PM media for about 2 weeks or until they were large enough to be placed into soil.

The data for GlgC16 Russet Burbank lines, including those expressing *GlgC16* from the patatin 1.0 (HS01; HS03; MT01), patatin 3.5 (HS13), and the small subunit of potato ADPGPP (HS10) promoters is presented below (Table 9). A number of *GlgC16* potato (variety Atlantic) lines were also examined (MT01; patatin 1.0 promoter). A processor would typically have to blanch to make acceptable products at a score of 2.0 or above. A number of Russet Burbank GlgC16 lines gave a fry score less than 2.0 immediately out of cold storage and thus could be processed directly. A fry color score of less than 2.0 is obtained with a large number of the lines after a very short period of reconditioning. This improved reconditioning response is seen for lines with increased solids and also for GlgC16 lines that did not show an increase in specific gravity. The improvement is also shown with all of these promoters used to express GlgC16 in the tuber. The effect of obtaining lines that may be fried directly out of storage and that recondition rapidly is also shown for

-29-

GlgC16 Atlantic. Line MT01-27 also demonstrated that increased starch in the tuber is not necessary to obtain enhanced cold storage properties since the specific gravity of the lines tested was not significantly different from that of the Atlantic control.

5

TABLE 9

Fry Color/Reconditioning response of potatoes containing GlgC16.

(Fry color rated according to USDA chart on a scale of 0- 4; lowest - highest).

		<u>Number of days at 65 °F</u>					
	<u>Line</u>	<u>0</u>	<u>3</u>	<u>6</u>	<u>10</u>	<u>13</u>	<u>17</u>
10	Control - Russet Burbank						
	RB02	2.2	2.3	1.8	2.0	1.0	1.3
	RB03	3.5	3.3	2.5	1.7	2.3	2.5
	RB05	2.3	2.5	2.2	2.0	1.2	1.3
	GlgC16 Russet Burbank						
15	HS13-47*	3.0	2.5	0.7	0.7	1.3	1.3
	HS10-15*	1.3	1.3	0.4	0.8	1.0	1.0
	HS13-50*	3.2	1.0	0.5	1.0	1.2	0.8
	MT01-82*	2.2	1.2	0.7	1.0	1.5	1.0
	HS13-36*	2.2	1.3	0.7	1.2	1.2	0.8
20	HS10-20*	1.0	0.7	0.5	0.5	0.4	0.5
	* - specific gravity greater than 1.083						
	HS01-58#	2.8	2.5	1.3	0.7	1.5	1.8
	HS03-20#	3.2	2.3	1.7	2.8	1.8	2.0
	HS03-18#	2.0	1.8	1.7	0.7	1.3	1.3
25	HS03-12A#	3.3	2.5	2.7	2.2	2.2	0.8
	HS03-12B#	3.0	3.2	2.0	2.7	1.3	2.0
	HS01-49#	2.2	1.3	2.3	2.2	1.5	0.8
	HS13-30#	3.2	2.3	2.0	2.8	1.7	3.0
	# - specific gravity less than 1.083						
30	Control - Atlantic						
	AT-1	2.3	2.3	2.3	1.5	1.5	1.8
	GlgC16 Atlantic						
	MT01-24	2.5	1.8	2.8	1.2	2.2	1.3
	MT01-27	1.0	0.5	1.3	0.3	0.8	0.7

35

-30-

EXAMPLE 3

The effect of GlgC16 on delaying sprouting was tested on a population of tubers stored at 60 °F (these tubers had been stored previously at 38-40 °F for 3 months). The tubers (4-6) were examined at intervals and 5 scored for the presence of sprouts of >0.5 cm (Table 10). The delay in sprouting, represented as the number of days to 50% sprouted, was frequently improved in the GlgC16 lines, was observed in the three varieties tested (Russet Burbank, Atlantic, and Norchip); was observed in lines where GlgC16 was expressed from the patatin 1.0 (HS01, HS03, and MT01), the patatin 3.5 10 (HS13), and the potato small ADPGPP (HS10) promoters; and was seen in lines with and without increased solids content. The lines with the delay sprouted normally when planted in soil.

Table 10

15	Variety	Line	Number of days to 50% sprouted
	Russet	Control	15
	Burbank	Control	14
		Control	9
		HS01-25	11
20		HS01-49	15
		HS01-58	18
		HS03-3	12
		HS03-5	13
		HS03-17	13
25		HS03-26	14
		HS03-27	12
		HS03-41	24
		HS10-10	14
		HS10-15	26
30		HS10-20	> 43†
		HS13-2	11
		HS13-13	16
		HS13-23	23
		HS13-30	15
35		HS13-34	25

-31-

	HS13-37	12
	HS13-47	21
	HS13-50	19
	HS13-68	13
5	HS13-70	24
	MT01-10	14
	MT01-11	13
	MT01-30	14
	MT01-37	19
10	MT01-82	16
Atlantic	Control	6
	MT01-6	11
	MT01-7	9
	MT01-15	10
15	MT01-31	6
Norchip	Control	8
	MT01-1	12
	MT01-5	13

† - duration of observation; tubers from this line sprouted when planted in soil.

20

EXAMPLE 4

Additional tests were performed with potatoes transformed with *glgC16* under the control of two different promoters. Promoters for the large subunit of potato tuber ADPGPP were isolated from two varieties of potato, Russet Burbank (SEQ ID NO:25) and Desiree (SEQ ID NO:26). The clones were identified using plaque hybridization with a probe from the 5' end of a cDNA from the large subunit of ADPglucose pyrophosphorylase. The translational start sites (ATG) of these clones were identified by plant consensus (Lutcke et al., 1987). PCR primers were used to introduce a BAMHI site at the 3' end downstream of the ATG and a HINDIII site at the 5' end of both promoters. The resulting 600 bp Russet Burbank promoter and 1600 bp Desiree promoters were ligated independently into pMON10098 in place of the E35S promoter, and fused with a BglII-SacI fragment from pMON20102 containing CTP-*glgC16* chimeric gene. The E35S-NPTII-Nos cassette was removed from these plasmids and replaced with a NotI- SalI fragment

containing the FMV-CTP-CP4-E9 cassette of pMON17227, discussed above, resulting in pMON21522 (Russet Burbank-derived promoter) and pMON21523 (Desiree-derived promoter). The pMON10098 plasmid contains the following DNA regions: 1) The chimeric kanamycin resistance gene engineered for plant expression to allow selection of the transformed tissue. The chimeric gene consists of the 0.35 Kb cauliflower mosaic virus 35S promoter (P-35S) (Odell et al., 1985), the 0.83 Kb NPTII gene, and the 0.26 Kb 3'-nontranslated region of the NOS 3'; 2) The 0.45 Kb ClaI to the DraI fragment from the pTi15955 octopine Ti plasmid, which contains the T-DNA left border region (Barker et al., 1983); 3) The 0.75 Kb segment containing the origin of replication from the RK2 plasmid (ori-V) (Stalker et al., 1981); 4) The 3.0 Kb SalI to PstI segment of pBR322 which provides the origin of replication for maintenance in *E. coli* (ori-322), and the *bom* site for the conjugational transfer into the *Agrobacterium tumefaciens* cells; 5) The 0.93 Kb fragment isolated from transposon Tn7 which encodes bacterial spectinomycin/streptomycin resistance (Spc/Str) (Fling et al., 1985), and is a determinant for selection in *E. coli* and *Agrobacterium tumefaciens*; 6) The 0.36 Kb PvuI to BclI fragment from the pTiT37 plasmid, which contains the nopaline-type T-DNA right border region (Fraley et al., 1985); and 7) The last segment is the expression cassette consisting of the 0.65 Kb cauliflower mosaic virus (CaMV) 35S promoter enhanced by duplication of the promoter sequence (P-E35S) (Kay et al., 1987), a synthetic multilinker with several unique cloning sites, and the 0.7 Kb 3' nontranslated region of the pea *rbcS-E9* gene (E9 3') (Coruzzi et al., 1984). The plasmid was mated into *Agrobacterium tumefaciens* strain ABI, using the triparental mating system, and used to transform Russet Burbank line Williams 82.

The improvements in storage characteristics have also been shown for Russet Burbank transformed with pMON22152 and pMON21523, in which GlgC16 is expressed from promoters for the large subunit of potato tuber ADPGPP. Field grown tubers were stored initially, after harvest for 1 month at 50°F, after which they were placed in cold storage at 40°F for 4 months. In one test, the fry color of fries produced from these tubers directly out of storage was evaluated by determining the reflectance of the fried material; lower values are preferred. In second test, a portion of the cold stored tubers were transferred to 55°F to determine the response in reconditioning. The data for these evaluations, for both the stem and bud ends of the tubers are presented in Table 11.

-33-

In both cases, many lines have better color values than the controls, both for direct frying and following reconditioning. For instance, pMON21522-144, pMON21523-79, and many others show dramatic improvements over the controls.

5

Table 11

pMON21522		<u>Reflectance of fried strips^{1,2}</u>			
		<u>40°F Storage³</u>		<u>40°F Storage: 21 d. @ 55°F⁴</u>	
LINE #		Bud ⁵	Stem ⁵	Bud	Stem
10 144		24.3	22.1	34.6	25.4
209		22.8	20.1	31.4	23.2
149		27.1	22.4	30.7	27.5
178		25.3	21.1	34.9	29.3
194		23.7	20.8	30.6	23.5
15 204		21.3	14.7	33.9	23.0
218		22.8	18.9	33.3	20.7
Control/Mean ⁶		19.7	16.8	31.0	25.1

pMON21523		<u>Reflectance of fried strips^{1,2}</u>			
		<u>40°F Storage³</u>		<u>40°F Storage: 21 d. @ 55°F⁴</u>	
LINE #		Bud ⁵	Stem ⁵	Bud	Stem
20 33		22.1	16.5	29.8	23.1
34		21.2	18.2	32.1	27.3
38		24.0	23.2	28.3	23.6
25 40		24.4	15.3	31.5	26.2
47		22.0	15.6	34.9	27.7
48		23.0	19.3	31.1	24.9
79		24.4	19.7	35.8	29.7
80		21.8	20.5	32.7	25.5
30 93		20.7	17.4	31.1	23.2
99		19.8	17.1	30.6	23.4
31		22.1	21.6	30.2	24.7
35		20.0	16.8	34.5	28.4
37		20.6	18.5	34.2	27.2
35 39		18.0	15.8	33.1	24.4

-34-

42	19.2	15.1	31.9	24.1
43	23.9	20.7	32.1	22.0
60	20.7	16.3	32.4	20.3
64	22.3	16.1	30.0	23.0
5 71	21.8	20.9	33.1	24.7
76	22.8	21.5	28.5	25.0
81	16.5	16.0	29.1	22.7
92	15.3	15.0	30.3	23.5
Control/Mean ⁶	19.7	16.8	31.0	25.1

10

¹ Four central strips were cut from each of 4-6 tubers and fried at 375°F.

² Reflectance measurements were taken using a PHOTOVOLT 577

Reflectance meter.

³ Strips were prepared from field-grown tubers that had been stored at 15 50°F for 1 month and then at 40°F for 4 months (cold storage)

⁴ Strips were prepared from field-grown tubers that had been stored at 50°F for 1 month and at 40°F for 4 months (cold storage), and subsequently reconditioned at 55°F for 21 days.

⁵ Reflectance was measured separately on bud and stem ends of fried 20 strips.

⁶ The mean values for 22 control Russet Burbank lines are presented for comparison.

From the foregoing, it will be seen that this invention is one well adapted to attain all the ends and objects hereinabove set forth together with 25 advantages which are obvious and which are inherent to the invention. It will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims. Since many possible embodiments may be made of the invention without departing from the scope 30 thereof, it is to be understood that all matter herein set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

REFERENCES

- Anderson, et al. (1989a) J. Biol. Chem. 264 (21):12238-12242.
- Anderson, et al. (1989b) First International Symposium on the Molecular Biology of the Potato, Bar Harbor, Maine.
- 5 ap Rees, et al. (1988) Symp. Soc. Exp. Biol. 42: 377-393.
- Baker, S.S., et al. (1994) Plant Mol. Biol. 24:701-713.
- Barker, et al. (1983) Plant Mol. Biol. 2:335-350.
- Bevan, et al. (1983) Nature (London) 304:184-187.
- Bevan, et al. (1986) Nucleic Acids Res. 14 (11):4625-4638.
- 10 Bhavé, et al. (1990) Plant Cell 2: 581-588.
- Birnboim, et al. (1979) Nucleic Acids Res. 7 :1513-1523.
- Blennow, et al. (1991) Phytochemistry 30:437-444.
- Burton, W.G. (1989) The Potato. Longman Scientific and Technical, Harlow, England, pp 365-522
- 15 Cattaneo, et al. (1969) Biochem. Biophys. Res. Commun. 34 (5):694- 701.
- Chang, et al. (1978) J. Bacteriol. 134; 1141-1156.
- Claassen, et al. (1991) Plant Physiol. 95: 1243-1249
- Coffin, et al. (1987) J. Food Sci. 52: 639-645.
- Copeland, L. and J. Preiss (1981) Plant Physiol. 68: 996-1001.
- 20 Coruzzi, et al. (1984) EMBO J. 3(8): 1671-1679.
- Creuzet-Sigal, et al. (1972) "Genetic Analysis and Biochemical Characterization of Mutants Impairing Glycogen Metabolism in *Escherichia coli* K12" in Biochemistry of the Glycosidic Linkage: An Integrated View. Edited by R. Piras and H. G. Pontis. 647-680. New York: Academic Press Inc.
- 25 Dickinson, D. B. and J. Preiss (1969) Plant Physiol. 44:1058-1062.
- Ditta, et al. (1980) Proc Natl Acad Sci USA 77, 7347-7351.
- Dixon, et al. (1981) Phytochemistry 20: 969-972.
- du Jardin, P. and Berhin, A. (1991) Plant Mol. Biol. 16:349-351.
- 30 Ebbelaar, et al. (1993) Int. Symp. on Gen. Manip. of Plant Metabolism and Growth, 29-31 March, Norwich UK Abstract #9.
- Ehrlich, H. A. (1989) Ed. PCR Technology - Principles and Applications for DNA Amplification. Stockton Press, New York.
- Fling, et al. (1985) Nucleic Acids Research 13 no. 19, 7095-7106.
- 35 Fraley, et al. (1983) Proc Natl Acad Sci USA 80, 4803-4807.

- Fraley, et al. (1985) *Bio/Technology* 3, 629-635.
- Frohman, M.A. (1990) In: *PCR Protocols*. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J., eds. Academic Press, San Diego, CA. pp. 28-38.
- 5 Frohman, et al. (1988) *Proc. Natl. Acad. Sci. USA* 85: 8998-9002.
- Ghosh, et al. (1966) *J. Biol. Chem.* 241 (19):4491-4504.
- Gilmour, et al. (1992) *Plant Mol. Biol.* 18:13-21.
- Hajela, et al. (1990) *Plant Physiol.* 93:1246-1252.
- Hannapel, D.J. (1990) *Plant Physiol.* 94: 919-925.
- 10 Hardenburg, et al. (1986) *USDA Agric. Handbook No 66*, pp 66-68
- Haugen, et al (1976) *J. Biol. Chem.* 251. (24) 7880-7885
- Heldt, et al. (1977) *Plant Physiol.* 59: 1146-1155.
- Houde, et al. (1992) *Plant Physiol.* 99(4):1381-1387.
- Huang, R.C., et al. (1987) *FED Proc.* 46 (6): 2238.
- 15 Iglesias, et al., (1993) *J. Biol Chem.* 268:1081-1086.
- Jarret, et al. (1980a) *Physiol. Plant.* 49: 177-184.
- Jarret, et al. (1980b) *J. Amer. Soc. Hort. Sci.* 105: 238-242.
- Jarret, et al. (1981) *In Vitro* 17: 825-830.
- Jefferson, et al. 1990. *Plant Mol. Biol.* 14: 995-1006.
- 20 Kaiser, W. M. and J. A. Bassham (1979) *Plant Physiol.* 63: 109-113.
- Kay, et al. (1987) *Science* 236:1299-1302.
- Koncz, C. and Schell, J. (1986) *Mol. Gen. Genet.* 204:383-396.
- Kossmann et al. (1991) *Mol. Gen. Genet.* 230:39-44.
- Krishnan, et al (1986) *Plant Physiol.* 81: 642-645.
- 25 Kruger, N.J. and Hammond, J.B.W. (1988) *Plant Physiol.* 86: 645-648.
- Kurkela, et al. (1990) *Plant Mol. Biol.* 15:137-144.
- Laemmli, U.K. (1970) *Nature (London)* 227:680-685.
- Leung, et al. (1986) *J. Bact.* 167 (1):82-88.
- Lin, et al. (1988a) *Plant Physiol.* 86 :1131-1135.
- 30 Lin, et al. (1988b) *Plant Physiol.* 88 :1175-1181.
- Liu, X. J., et al. (1990) *Mol. Gen. Genet.* 223:401-406.
- Loh, et al. (1989) *Science* 243:217-220.
- Lutcke et al. (1987) *EMBO J.* 6(1):43-48.
- Mignery, et al. (1988) *Gene* 62 :27-44.
- 35 Miller, J. H. (1972) *Experiments in Molecular Genetics*. Cold Spring

Harbor Laboratory, Cold Spring Harbor, New York.

- Miner, et al. (1992) J. Neurosci. Res. 33(1): 10-18.
- Morell, et al. (1988) J. Biol. Chem. 263:633-637.
- Morell, et al. (1987) Plant Physiol. 85 :182-187.
- 5 Mori et al. (1991) J. Biol. Chem. 266: 18446-18453.
- Muller, et al. (1990) Mol. Gen. Genet. 224:136-146.
- Murata, et al. (1992) Nature 356:710-713.
- Nakano et al. (1989) J. Biochem. 106: 691-695.
- Nakata, et al. (1992) J. Cell. Biochem. Suppl. 16F, Abst. Y311, p.266.
- 10 Nordin, K., et al. (1993) Plant Mol. Biol. 21: 641-653.
- Odell, et al. (1985) Nature 313, 810-812.
- Ohta et al. (1991) Mol. Gen. Genet. 225: 369-378.
- Okita, et al. (1990) 93: 785-790.
- Olive, et al. (1989) Plant Mol. Biol. 12: 525-538.
- 15 Plaxton, et al. (1987) Plant Physiol. 83: 105-112.
- Preiss, Jack. (1988) "Biosynthesis of Starch and Its Regulation" in The Biochemistry of Plants. Edited by J. Preiss. 184-249. Orlando, FL: Academic Press.
- Preiss, et al. (1971) Biochem. Biophys. Res. Comm. 42:180-186.
- 20 Pressey, R. (1966) Arch. Biochem. Biophys. 113: 667-674.
- Qoronfleh, et al. (1992) J. Bacteriol. 174(24): 7902-7909.
- Rocha-Sosa, et al. (1989) EMBO J. 8(1):23-29.
- Rohde et al., (1990) J. Genet. & Breed. 44:311-315.
- Ryall, A.L. and Lipton, W.J. (1979) Vegetables and Melons, Vol. 1. AVI, Westport, CT, pp 225-230, 272-279.
- 25 Sambrook, et al. (1989) Molecular Cloning, A Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory Press, N.Y.
- Santarius, et al. (1965) Biochim. Biophys. Acta 102:39-54.
- Shahar et al. (1992) Plant Cell 4:135-147.
- 30 Shallenberger, et al. (1959) Agric. and Food Chem. 7: 274-277.
- Smith-White, B. and J. Preiss (1992) J. Mol. Evol. 34:449-464.
- Solanoubat, M. and G. Belliard (1987) Geneg 60:47-56.
- Solanoubat, M. and G. Belliard (1989) Geneg 84:181-185.
- Sowokinos, J. R., and J. Preiss. (1982) Plant Physiol. 69:1459-1466.
- 35 Stalker, et al (1981) Mol Gen Genet 181, 8-12.

- Stiekema et al. (1988) *Plant Mol. Biol.* 11: 255-269.
- Stukerlj et al. (1990) *Nucl. Acids Res.* 18:46050.
- Svab, et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:8526-8530.
- Takaha et al., (1993) *J. Biol. Chem.* 268:1391-1396.
- 5 Timko, et al. (1985) *Nature (London)* 318: 579-582.
- Tsai, C. Y. and O. E. Nelson. (1966) *Science* 151: 341-343.
- Vasil, V., F. Redway and I. Vasil. (1990) *Bio/Technology* 8:429-434.
- van Berkel, J., et al. (1991) Abstract # 1576 presented at the International Congress of Plant Molecular Biology, Tucson, AZ.
- 10 van Berkel, J., et al. (1994) *Plant Physiol.* 104: 445-452.
- Von Scheele, C. (1937) *Landw Ver Stn* 127: 67-96.
- Weaver, et al. (1978) *Am. Pot. J.* 55:83-93.
- White, T.C., et al. (1992) *Plant Physiol.* 99 (Suppl. 1):78.
- Wilhelm, K. S., et al. (1993) *Plant Mol. Biol.* 23: 1073-1077.
- 15 Wong, et al. (1988) *Geneg* 68: 193-203.
- Yamaguchi-Shinozaki, et al. (1993) *Mol. Gen. Genet.* 238:331-340.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (1994) *The Plant Cell* 6: 251-264.
- Yoshida et al. (1992) *Geneg* 10: 255-259.
- 20 Zhu, B., et al. (1993) *Plant Mol. Biol.* 21: 729-735.

-39-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Monsanto Company
 (B) STREET: 800 North Lindbergh
 (C) CITY: St. Louis
 (D) STATE: Missouri
 (E) COUNTRY: United States of America
 (F) POSTAL CODE (ZIP): 63167
 (G) TELEPHONE: (314)694-3131
 (H) TELEFAX: (314)694-5435

(ii) TITLE OF INVENTION: Method of Improving the Quality of Stored Potatoes

(iii) NUMBER OF SEQUENCES: 26

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/070155
 (B) FILING DATE: 28-MAY-1993

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1296 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..1296

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GTT AGT TTA GAG AAG AAC GAT CAC TTA ATG TTG GCG CGC CAG CTG	48
Met Val Ser Leu Glu Lys Asn Asp His Leu Met Leu Ala Arg Gln Leu	
1 5 10 15	
CCA TTG AAA TCT GTT GCC CTG ATA CTG GCG GGA GGA CGT GGT ACC CGC	96
Pro Leu Lys Ser Val Ala Leu Ile Leu Ala Gly Gly Arg Gly Thr Arg	
20 25 30	
CTG AAG GAT TTA ACC AAT AAG CGA GCA AAA CCG GCC GTA CAC TTC GGC	144
Leu Lys Asp Leu Thr Asn Lys Arg Ala Lys Pro Ala Val His Phe Gly	
35 40 45	
GGT AAG TTC CGC ATT ATC GAC TTT GCG CTG TCT AAC TGC ATC AAC TCC	192
Gly Lys Phe Arg Ile Ile Asp Phe Ala Leu Ser Asn Cys Ile Asn Ser	
50 55 60	

GGG	ATC	CGT	CGT	ATG	GGC	GTG	ATC	ACC	CAG	TAC	CAG	TCC	CAC	ACT	CTG	240
Gly	Ile	Arg	Arg	Met	Gly	Val	Ile	Thr	Gln	Tyr	Gln	Ser	His	Thr	Leu	
65					70				75						80	
GTG	CAG	CAC	ATT	CAG	CGC	GGC	TGG	TCA	TTC	TTC	AAT	GAA	GAA	ATG	AAC	288
Val	Gln	His	Ile	Gln	Arg	Gly	Trp	Ser	Phe	Phe	Asn	Glu	Glu	Met	Asn	
				85					90					95		
GAG	TTT	GTC	GAT	CTG	CTG	CCA	GCA	CAG	CAG	AGA	ATG	AAA	GGG	GAA	AAC	336
Glu	Phe	Val	Asp	Leu	Leu	Pro	Ala	Gln	Gln	Arg	Met	Lys	Gly	Glu	Asn	
			100					105					110			
TGG	TAT	CGC	GGC	ACC	GCA	GAT	GCG	GTC	ACC	CAA	AAC	CTC	GAC	ATT	ATC	384
Trp	Tyr	Arg	Gly	Thr	Ala	Asp	Ala	Val	Thr	Gln	Asn	Leu	Asp	Ile	Ile	
			115				120					125				
CGT	CGT	TAT	AAA	GCG	GAA	TAC	GTG	GTG	ATC	CTG	GCG	GGC	GAC	CAT	ATC	432
Arg	Arg	Tyr	Lys	Ala	Glu	Tyr	Val	Val	Ile	Leu	Ala	Gly	Asp	His	Ile	
	130					135					140					
TAC	AAG	CAA	GAC	TAC	TCG	CGT	ATG	CTT	ATC	GAT	CAC	GTC	GAA	AAA	GGT	480
Tyr	Lys	Gln	Asp	Tyr	Ser	Arg	Met	Leu	Ile	Asp	His	Val	Glu	Lys	Gly	
	145				150					155					160	
GTA	CGT	TGT	ACC	GTT	GTT	TGT	ATG	CCA	GTA	CCG	ATT	GAA	GAA	GCC	TCC	528
Val	Arg	Cys	Thr	Val	Val	Cys	Met	Pro	Val	Pro	Ile	Glu	Glu	Ala	Ser	
				165					170					175		
GCA	TTT	GGC	GTT	ATG	GCG	GTT	GAT	GAG	AAC	GAT	AAA	ACT	ATC	GAA	TTC	576
Ala	Phe	Gly	Val	Met	Ala	Val	Asp	Glu	Asn	Asp	Lys	Thr	Ile	Glu	Phe	
			180					185					190			
GTG	GAA	AAA	CCT	GCT	AAC	CCG	CCG	TCA	ATG	CCG	AAC	GAT	CCG	AGC	AAA	624
Val	Glu	Lys	Pro	Ala	Asn	Pro	Pro	Ser	Met	Pro	Asn	Asp	Pro	Ser	Lys	
		195					200					205				
TCT	CTG	GCG	AGT	ATG	GGT	ATC	TAC	GTC	TTT	GAC	GCC	GAC	TAT	CTG	TAT	672
Ser	Leu	Ala	Ser	Met	Gly	Ile	Tyr	Val	Phe	Asp	Ala	Asp	Tyr	Leu	Tyr	
	210					215					220					
GAA	CTG	CTG	GAA	GAA	GAC	GAT	CGC	GAT	GAG	AAC	TCC	AGC	CAC	GAC	TTT	720
Glu	Leu	Leu	Glu	Glu	Asp	Asp	Arg	Asp	Glu	Asn	Ser	Ser	His	Asp	Phe	
	225				230					235					240	
GGC	AAA	GAT	TTG	ATT	CCC	AAG	ATC	ACC	GAA	GCC	GGT	CTG	GCC	TAT	GCG	768
Gly	Lys	Asp	Leu	Ile	Pro	Lys	Ile	Thr	Glu	Ala	Gly	Leu	Ala	Tyr	Ala	
				245					250					255		
CAC	CCG	TTC	CCG	CTC	TCT	TGC	GTA	CAA	TCC	GAC	CCG	GAT	GCC	GAG	CCG	816
His	Pro	Phe	Pro	Leu	Ser	Cys	Val	Gln	Ser	Asp	Pro	Asp	Ala	Glu	Pro	
			260					265					270			
TAC	TGG	CGC	GAT	GTG	GGT	ACG	CTG	GAA	GCT	TAC	TGG	AAA	GCG	AAC	CTC	864
Tyr	Trp	Arg	Asp	Val	Gly	Thr	Leu	Glu	Ala	Tyr	Trp	Lys	Ala	Asn	Leu	
		275					280					285				
GAT	CTG	GCC	TCT	GTG	GTG	CCG	AAA	CTG	GAT	ATG	TAC	GAT	CGC	AAT	TGG	912
Asp	Leu	Ala	Ser	Val	Val	Pro	Lys	Leu	Asp	Met	Tyr	Asp	Arg	Asn	Trp	
	290					295					300					
CCA	ATT	CGC	ACC	TAC	AAT	GAA	TCA	TTA	CCG	CCA	GCG	AAA	TTC	GTG	CAG	960
Pro	Ile	Arg	Thr	Tyr	Asn	Glu	Ser	Leu	Pro	Pro	Ala	Lys	Phe	Val	Gln	
	305				310					315					320	

-41-

GAT CGC TCC GGT AGC CAC GGG ATG ACC CTT AAC TCA CTG GTT TCC GGC	1008
Asp Arg Ser Gly Ser His Gly Met Thr Leu Asn Ser Leu Val Ser Gly	
325 330 335	
GGT TGT GTG ATC TCC GGT TCG GTG GTG GTG CAG TCC GTT CTG TTC TCG	1056
Gly Cys Val Ile Ser Gly Ser Val Val Val Gln Ser Val Leu Phe Ser	
340 345 350	
CGC GTT CGC GTG AAT TCA TTC TGC AAC ATT GAT TCC GCC GTA TTG TTA	1104
Arg Val Arg Val Asn Ser Phe Cys Asn Ile Asp Ser Ala Val Leu Leu	
355 360 365	
CCG GAA GTA TGG GTA GGT CGC TCG TGC CGT CTG CGC CGC TGC GTC ATC	1152
Pro Glu Val Trp Val Gly Arg Ser Cys Arg Leu Arg Arg Cys Val Ile	
370 375 380	
GAT CGT GCT TGT GTT ATT CCG GAA GGC ATG GTG ATT GGT GAA AAC GCA	1200
Asp Arg Ala Cys Val Ile Pro Glu Gly Met Val Ile Gly Glu Asn Ala	
385 390 395 400	
GAG GAA GAT GCA CGT CGT TTC TAT CGT TCA GAA GAA GGC ATC GTG CTG	1248
Glu Glu Asp Ala Arg Arg Phe Tyr Arg Ser Glu Glu Gly Ile Val Leu	
405 410 415	
GTA ACG CGC GAA ATG CTA CGG AAG TTA GGG CAT AAA CAG GAG CGA TAA	1296
Val Thr Arg Glu Met Leu Arg Lys Leu Gly His Lys Gln Glu Arg	
420 425 430	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 431 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Val Ser Leu Glu Lys Asn Asp His Leu Met Leu Ala Arg Gln Leu	
1 5 10 15	
Pro Leu Lys Ser Val Ala Leu Ile Leu Ala Gly Gly Arg Gly Thr Arg	
20 25 30	
Leu Lys Asp Leu Thr Asn Lys Arg Ala Lys Pro Ala Val His Phe Gly	
35 40 45	
Gly Lys Phe Arg Ile Ile Asp Phe Ala Leu Ser Asn Cys Ile Asn Ser	
50 55 60	
Gly Ile Arg Arg Met Gly Val Ile Thr Gln Tyr Gln Ser His Thr Leu	
65 70 75 80	
Val Gln His Ile Gln Arg Gly Trp Ser Phe Phe Asn Glu Glu Met Asn	
85 90 95	
Glu Phe Val Asp Leu Leu Pro Ala Gln Gln Arg Met Lys Gly Glu Asn	
100 105 110	
Trp Tyr Arg Gly Thr Ala Asp Ala Val Thr Gln Asn Leu Asp Ile Ile	
115 120 125	

-42-

Arg Arg Tyr Lys Ala Glu Tyr Val Val Ile Leu Ala Gly Asp His Ile
 130 135 140
 Tyr Lys Gln Asp Tyr Ser Arg Met Leu Ile Asp His Val Glu Lys Gly
 145 150 155 160
 Val Arg Cys Thr Val Val Cys Met Pro Val Pro Ile Glu Glu Ala Ser
 165 170 175
 Ala Phe Gly Val Met Ala Val Asp Glu Asn Asp Lys Thr Ile Glu Phe
 180 185 190
 Val Glu Lys Pro Ala Asn Pro Pro Ser Met Pro Asn Asp Pro Ser Lys
 195 200 205
 Ser Leu Ala Ser Met Gly Ile Tyr Val Phe Asp Ala Asp Tyr Leu Tyr
 210 215 220
 Glu Leu Leu Glu Glu Asp Asp Arg Asp Glu Asn Ser Ser His Asp Phe
 225 230 235 240
 Gly Lys Asp Leu Ile Pro Lys Ile Thr Glu Ala Gly Leu Ala Tyr Ala
 245 250 255
 His Pro Phe Pro Leu Ser Cys Val Gln Ser Asp Pro Asp Ala Glu Pro
 260 265 270
 Tyr Trp Arg Asp Val Gly Thr Leu Glu Ala Tyr Trp Lys Ala Asn Leu
 275 280 285
 Asp Leu Ala Ser Val Val Pro Lys Leu Asp Met Tyr Asp Arg Asn Trp
 290 295 300
 Pro Ile Arg Thr Tyr Asn Glu Ser Leu Pro Pro Ala Lys Phe Val Gln
 305 310 315 320
 Asp Arg Ser Gly Ser His Gly Met Thr Leu Asn Ser Leu Val Ser Gly
 325 330 335
 Gly Cys Val Ile Ser Gly Ser Val Val Val Gln Ser Val Leu Phe Ser
 340 345 350
 Arg Val Arg Val Asn Ser Phe Cys Asn Ile Asp Ser Ala Val Leu Leu
 355 360 365
 Pro Glu Val Trp Val Gly Arg Ser Cys Arg Leu Arg Arg Cys Val Ile
 370 375 380
 Asp Arg Ala Cys Val Ile Pro Glu Gly Met Val Ile Gly Glu Asn Ala
 385 390 395 400
 Glu Glu Asp Ala Arg Arg Phe Tyr Arg Ser Glu Glu Gly Ile Val Leu
 405 410 415
 Val Thr Arg Glu Met Leu Arg Lys Leu Gly His Lys Gln Glu Arg
 420 425 430

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1296 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-43-

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1296

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG GTT AGT TTA GAG AAG AAC GAT CAC TTA ATG TTG GCG CGC CAG CTG	48
Met Val Ser Leu Glu Lys Asn Asp His Leu Met Leu Ala Arg Gln Leu	
1 5 10 15	
CCA TTG AAA TCT GTT GCC CTG ATA CTG GCG GGA GGA CGT GGT ACC CGC	96
Pro Leu Lys Ser Val Ala Leu Ile Leu Ala Gly Gly Arg Gly Thr Arg	
20 25 30	
CTG AAG GAT TTA ACC AAT AAG CGA GCA AAA CCG GCC GTA CAC TTC GGC	144
Leu Lys Asp Leu Thr Asn Lys Arg Ala Lys Pro Ala Val His Phe Gly	
35 40 45	
GGT AAG TTC CGC ATT ATC GAC TTT GCG CTG TCT AAC TGC ATC AAC TCC	192
Gly Lys Phe Arg Ile Ile Asp Phe Ala Leu Ser Asn Cys Ile Asn Ser	
50 55 60	
GGG ATC CGT CGT ATG GGC GTG ATC ACC CAG TAC CAG TCC CAC ACT CTG	240
Gly Ile Arg Arg Met Gly Val Ile Thr Gln Tyr Gln Ser His Thr Leu	
65 70 75 80	
GTG CAG CAC ATT CAG CGC GGC TGG TCA TTC TTC AAT GAA GAA ATG AAC	288
Val Gln His Ile Gln Arg Gly Trp Ser Phe Phe Asn Glu Glu Met Asn	
85 90 95	
GAG TTT GTC GAT CTG CTG CCA GCA CAG CAG AGA ATG AAA GGG GAA AAC	336
Glu Phe Val Asp Leu Leu Pro Ala Gln Gln Arg Met Lys Gly Glu Asn	
100 105 110	
TGG TAT CGC GGC ACC GCA GAT GCG GTC ACC CAA AAC CTC GAC ATT ATC	384
Trp Tyr Arg Gly Thr Ala Asp Ala Val Thr Gln Asn Leu Asp Ile Ile	
115 120 125	
CGT CGT TAT AAA GCG GAA TAC GTG GTG ATC CTG GCG GGC GAC CAT ATC	432
Arg Arg Tyr Lys Ala Glu Tyr Val Val Ile Leu Ala Gly Asp His Ile	
130 135 140	
TAC AAG CAA GAC TAC TCG CGT ATG CTT ATC GAT CAC GTC GAA AAA GGT	480
Tyr Lys Gln Asp Tyr Ser Arg Met Leu Ile Asp His Val Glu Lys Gly	
145 150 155 160	
GTA CGT TGT ACC GTT GTT TGT ATG CCA GTA CCG ATT GAA GAA GCC TCC	528
Val Arg Cys Thr Val Val Cys Met Pro Val Pro Ile Glu Glu Ala Ser	
165 170 175	
GCA TTT GGC GTT ATG GCG GTT GAT GAG AAC GAT AAA ACT ATC GAA TTC	576
Ala Phe Gly Val Met Ala Val Asp Glu Asn Asp Lys Thr Ile Glu Phe	
180 185 190	
GTG GAA AAA CCT GCT AAC CCG CCG TCA ATG CCG AAC GAT CCG AGC AAA	624
Val Glu Lys Pro Ala Asn Pro Pro Ser Met Pro Asn Asp Pro Ser Lys	
195 200 205	
TCT CTG GCG AGT ATG GGT ATC TAC GTC TTT GAC GCC GAC TAT CTG TAT	672
Ser Leu Ala Ser Met Gly Ile Tyr Val Phe Asp Ala Asp Tyr Leu Tyr	
210 215 220	

GAA CTG CTG GAA GAA GAC GAT CGC GAT GAG AAC TCC AGC CAC GAC TTT Glu Leu Leu Glu Glu Asp Asp Arg Asp Glu Asn Ser Ser His Asp Phe 225 230 235 240	720
GGC AAA GAT TTG ATT CCC AAG ATC ACC GAA GCC GGT CTG GCC TAT GCG Gly Lys Asp Leu Ile Pro Lys Ile Thr Glu Ala Gly Leu Ala Tyr Ala 245 250 255	768
CAC CCG TTC CCG CTC TCT TGC GTA CAA TCC GAC CCG GAT GCC GAG CCG His Pro Phe Pro Leu Ser Cys Val Gln Ser Asp Pro Asp Ala Glu Pro 260 265 270	816
TAC TGG CGC GAT GTG GGT ACG CTG GAA GCT TAC TGG AAA GCG AAC CTC Tyr Trp Arg Asp Val Gly Thr Leu Glu Ala Tyr Trp Lys Ala Asn Leu 275 280 285	864
GAT CTG GCC TCT GTG GTG CCG GAA CTG GAT ATG TAC GAT CGC AAT TGG Asp Leu Ala Ser Val Val Pro Glu Leu Asp Met Tyr Asp Arg Asn Trp 290 295 300	912
CCA ATT CGC ACC TAC AAT GAA TCA TTA CCG CCA GCG AAA TTC GTG CAG Pro Ile Arg Thr Tyr Asn Glu Ser Leu Pro Pro Ala Lys Phe Val Gln 305 310 315 320	960
GAT CGC TCC GGT AGC CAC GGG ATG ACC CTT AAC TCA CTG GTT TCC GAC Asp Arg Ser Gly Ser His Gly Met Thr Leu Asn Ser Leu Val Ser Asp 325 330 335	1008
GGT TGT GTG ATC TCC GGT TCG GTG GTG GTG CAG TCC GTT CTG TTC TCG Gly Cys Val Ile Ser Gly Ser Val Val Val Gln Ser Val Leu Phe Ser 340 345 350	1056
CGC GTT CGC GTG AAT TCA TTC TGC AAC ATT GAT TCC GCC GTA TTG TTA Arg Val Arg Val Asn Ser Phe Cys Asn Ile Asp Ser Ala Val Leu Leu 355 360 365	1104
CCG GAA GTA TGG GTA GGT CGC TCG TGC CGT CTG CGC CGC TGC GTC ATC Pro Glu Val Trp Val Gly Arg Ser Cys Arg Leu Arg Arg Cys Val Ile 370 375 380	1152
GAT CGT GCT TGT GTT ATT CCG GAA GGC ATG GTG ATT GGT GAA AAC GCA Asp Arg Ala Cys Val Ile Pro Glu Gly Met Val Ile Gly Glu Asn Ala 385 390 395 400	1200
GAG GAA GAT GCA CGT CGT TTC TAT CGT TCA GAA GAA GGC ATC GTG CTG Glu Glu Asp Ala Arg Arg Phe Tyr Arg Ser Glu Glu Gly Ile Val Leu 405 410 415	1248
GTA ACG CGC GAA ATG CTA CGG AAG TTA GGG CAT AAA CAG GAG CGA TAA Val Thr Arg Glu Met Leu Arg Lys Leu Gly His Lys Gln Glu Arg 420 425 430	1296

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

-45-

Met Val Ser Leu Glu Lys Asn Asp His Leu Met Leu Ala Arg Gln Leu
 1 5 10 15
 Pro Leu Lys Ser Val Ala Leu Ile Leu Ala Gly Gly Arg Gly Thr Arg
 20 25 30
 Leu Lys Asp Leu Thr Asn Lys Arg Ala Lys Pro Ala Val His Phe Gly
 35 40 45
 Gly Lys Phe Arg Ile Ile Asp Phe Ala Leu Ser Asn Cys Ile Asn Ser
 50 55 60
 Gly Ile Arg Arg Met Gly Val Ile Thr Gln Tyr Gln Ser His Thr Leu
 65 70 75 80
 Val Gln His Ile Gln Arg Gly Trp Ser Phe Phe Asn Glu Glu Met Asn
 85 90 95
 Glu Phe Val Asp Leu Leu Pro Ala Gln Gln Arg Met Lys Gly Glu Asn
 100 105 110
 Trp Tyr Arg Gly Thr Ala Asp Ala Val Thr Gln Asn Leu Asp Ile Ile
 115 120 125
 Arg Arg Tyr Lys Ala Glu Tyr Val Val Ile Leu Ala Gly Asp His Ile
 130 135 140
 Tyr Lys Gln Asp Tyr Ser Arg Met Leu Ile Asp His Val Glu Lys Gly
 145 150 155 160
 Val Arg Cys Thr Val Val Cys Met Pro Val Pro Ile Glu Glu Ala Ser
 165 170 175
 Ala Phe Gly Val Met Ala Val Asp Glu Asn Asp Lys Thr Ile Glu Phe
 180 185 190
 Val Glu Lys Pro Ala Asn Pro Pro Ser Met Pro Asn Asp Pro Ser Lys
 195 200 205
 Ser Leu Ala Ser Met Gly Ile Tyr Val Phe Asp Ala Asp Tyr Leu Tyr
 210 215 220
 Glu Leu Leu Glu Glu Asp Asp Arg Asp Glu Asn Ser Ser His Asp Phe
 225 230 235 240
 Gly Lys Asp Leu Ile Pro Lys Ile Thr Glu Ala Gly Leu Ala Tyr Ala
 245 250 255
 His Pro Phe Pro Leu Ser Cys Val Gln Ser Asp Pro Asp Ala Glu Pro
 260 265 270
 Tyr Trp Arg Asp Val Gly Thr Leu Glu Ala Tyr Trp Lys Ala Asn Leu
 275 280 285
 Asp Leu Ala Ser Val Val Pro Glu Leu Asp Met Tyr Asp Arg Asn Trp
 290 295 300
 Pro Ile Arg Thr Tyr Asn Glu Ser Leu Pro Pro Ala Lys Phe Val Gln
 305 310 315 320
 Asp Arg Ser Gly Ser His Gly Met Thr Leu Asn Ser Leu Val Ser Asp
 325 330 335

-46-

Gly Cys Val Ile Ser Gly Ser Val Val Gln Ser Val Leu Phe Ser
340 345 350

Arg Val Arg Val Asn Ser Phe Cys Asn Ile Asp Ser Ala Val Leu Leu
355 360 365

Pro Glu Val Trp Val Gly Arg Ser Cys Arg Leu Arg Arg Cys Val Ile
370 375 380

Asp Arg Ala Cys Val Ile Pro Glu Gly Met Val Ile Gly Glu Asn Ala
385 390 395 400

Glu Glu Asp Ala Arg Arg Phe Tyr Arg Ser Glu Glu Gly Ile Val Leu
405 410 415

Val Thr Arg Glu Met Leu Arg Lys Leu Gly His Lys Gln Glu Arg
420 425 430

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 355 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) **FEATURE:**

- (A) NAME/KEY: CDS
(B) LOCATION: 88..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGCTTG TTC TCATTGTTGT TATCATTATA TATAGATGAC CAAAGCACTA GACCAAACCT 60

CAGTCACACA AAGAGTAAAG AAGAACA ATG GCT TCC TCT ATG CTC TCT TCC 111
Met Ala Ser Ser Met Leu Ser Ser
1 5

GCT ACT ATG GTT GCC TCT CCG GCT CAG GCC ACT ATG GTC GCT CCT TTC 159
Ala Thr Met Val Ala Ser Pro Ala Gln Ala Thr Met Val Ala Pro Phe
10 15 20

AAC GGA CTT AAG TCC TCC GCT GCC TTC CCA GCC ACC CGC AAG GCT AAC 207
Asn Gly Leu Lys Ser Ser Ala Ala Phe Pro Ala Thr Arg Lys Ala Asn
25 30 35 40

AAC GAC ATT ACT TCC ATC ACA AGC AAC GGC GGA AGA GTT AAC TGC ATG 255
Asn Asp Ile Thr Ser Ile Thr Ser Asn Gly Gly Arg Val Asn Cys Met
45 50 55

-47-

CAG T TGG CCT CCG ATT GGA AAG AAG AAG TTT GAG ACT CTC TCT TAC	303
Gln Val Trp Pro Pro Ile Gly Lys Lys Lys Phe Glu Thr Leu Ser Tyr	
60 65 70	
CTT CCT GAC CTT ACC GAT TCC GGT GGT CGC GTC AAC TGC ATG CAG GCC	351
Leu Pro Asp Leu Thr Asp Ser Gly Gly Arg Val Asn Cys Met Gln Ala	
75 80 85	
ATG G	355
Met	

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Ser Ser Met Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala	
1 5 10 15	
Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala	
20 25 30	
Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser	
35 40 45	
Asn Gly Gly Arg Val Asn Cys Met Gln Val Trp Pro Pro Ile Gly Lys	
50 55 60	
Lys Lys Phe Glu Thr Leu Ser Tyr Leu Pro Asp Leu Thr Asp Ser Gly	
65 70 75 80	
Gly Arg Val Asn Cys Met Gln Ala Met	
85	

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1575 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..1565

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CC ATG GCG GCT TCC ATT GGA GCC TTA AAA TCT TCA CCT TCT TCT AAC	47
Met Ala Ala Ser Ile Gly Ala Leu Lys Ser Ser Pro Ser Ser Asn	
1 5 10 15	
AAT TGC ATC AAT GAG AGA AGA AAT GAT TCT ACA CGT GCT GTA TCC AGC	95
Asn Cys Ile Asn Glu Arg Arg Asn Asp Ser Thr Arg Ala Val Ser Ser	
20 25 30	
AGA AAT CTC TCA TTT TCG TCT TCT CAT CTC GCC GGA GAC AAG TTG ATG	143
Arg Asn Leu Ser Phe Ser Ser Ser His Leu Ala Gly Asp Lys Leu Met	
35 40 45	
CCT GTA TCG TCC TTA CGT TCC CAA GGA GTC CGA TTC AAT GTG AGA AGA	191
Pro Val Ser Ser Leu Arg Ser Gln Gly Val Arg Phe Asn Val Arg Arg	
50 55 60	
AGT CCA ATG ATT GTG TCG CCA AAG GCT GTT TCT GAT TCG CAG AAT TCA	239
Ser Pro Met Ile Val Ser Pro Lys Ala Val Ser Asp Ser Gln Asn Ser	
65 70 75	
CAG ACA TGT CTA GAC CCA GAT GCT AGC CGG AGT GTT TTG GGA ATT ATT	287
Gln Thr Cys Leu Asp Pro Asp Ala Ser Arg Ser Val Leu Gly Ile Ile	
80 85 90 95	
CTT GGA GGT GGA GCT GGG ACC CGA CTT TAT CCT CTA ACT AAA AAA AGA	335
Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg	
100 105 110	
GCA AAG CCA GCT GTT CCA CTT GGA GCA AAT TAT CGT CTG ATT GAC ATT	383
Ala Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile	
115 120 125	
CCT GTA AGC AAC TGC TTG AAC AGT AAT ATA TCC AAG ATT TAT GTT CTC	431
Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu	
130 135 140	
ACA CAA TTC AAC TCT GCC TCT CTG AAT CGC CAC CTT TCA CGA GCA TAT	479
Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr	
145 150 155	
GCT AGC AAC ATG GGA GGA TAC AAA AAC GAG GGC TTT GTG GAA GTT CTT	527
Ala Ser Asn Met Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val Leu	
160 165 170 175	
GCT GCT CAA CAA AGT CCA GAG AAC CCC GAT TGG TTC CAG GGC ACG GCT	575
Ala Ala Gln Gln Ser Pro Glu Asn Pro Asp Trp Phe Gln Gly Thr Ala	
180 185 190	
GAT GCT GTC AGA CAA TAT CTG TGG TTG TTT GAG GAG CAT ACT GTT CTT	623
Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Thr Val Leu	
195 200 205	

-49-

GAA TAC CTT ATA CTT GCT GGA GAT CAT CTG TAT CGA ATG GAT TAT GAA Glu Tyr Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu 210 215 220	671
AAG TTT ATT CAA GCC CAC AGA GAA ACA GAT GCT GAT ATT ACC GTT GCC Lys Phe Ile Gln Ala His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala 225 230 235	719
GCA CTG CCA ATG GAC GAG AAG CGT GCC ACT GCA TTC GGT CTC ATG AAG Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met Lys 240 245 250 255	767
ATT GAC GAA GAA GGA CGC ATT ATT GAA TTT GCA GAG AAA CCG CAA GGA Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln Gly 260 265 270	815
GAG CAA TTG CAA GCA ATG AAA GTG GAT ACT ACC ATT TTA GGT CTT GAT Glu Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu Asp 275 280 285	863
GAC AAG AGA GCT AAA GAA ATG CCT TTC ATT GCC AGT ATG GGT ATA TAT Asp Lys Arg Ala Lys Glu Met Pro Phe Ile Ala Ser Met Gly Ile Tyr 290 295 300	911
GTC ATT AGC AAA GAC GTG ATG TTA AAC CTA CTT CGT GAC AAG TTC CCT Val Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe Pro 305 310 315	959
GGG GCC AAT GAT TTT GGT AGT GAA GTT ATT CCT GGT GCA ACT TCA CTT Gly Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser Leu 320 325 330 335	1007
GGG ATG AGA GTG CAA GCT TAT TTA TAT GAT GGG TAC TGG GAA GAT ATT Gly Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile 340 345 350	1055
GGT ACC ATT GAA GCT TTC TAC AAT GCC AAT TTG GGC ATT ACA AAA AAG Gly Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys Lys 355 360 365	1103
CCG GTG CCA GAT TTT AGC TTT TAC GAC CGA TCA GCC CCA ATC TAC ACC Pro Val Pro Asp Phe Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr 370 375 380	1151
CAA CCT CGA TAT CTA CCA CCA TCA AAA ATG CTT GAT GCT GAT GTC ACA Gln Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val Thr 385 390 395	1199
GAT AGT GTC ATT GGT GAA GGT TGT GTG ATC AAG AAC TGT AAG ATT CAT Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His 400 405 410 415	1247
CAT TCC GTG GTT GGA CTC AGA TCA TGC ATA TCA GAG GGA GCA ATT ATA His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile 420 425 430	1295

-50-

GAA GAC TCA CTT TTG ATG GGG GCA GAT TAC TAT GAG ACT GAT GCT GAC	1343
Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala Asp	
435 440 445	
AGG AAG TTG CTG GCT GCA AAG GGC AGT GTC CCA ATT GGC ATC GGC AAG	1391
Arg Lys Leu Leu Ala Ala Lys Gly Ser Val Pro Ile Gly Ile Gly Lys	
450 455 460	
AAT TGT CAC ATT AAA AGA GCC ATT ATC GAC AAG AAT GCC CGT ATA GGG	1439
Asn Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly	
465 470 475	
GAC AAT GTG AAG ATC ATT AAC AAA GAC AAC GTT CAA GAA GCG GCT AGG	1487
Asp Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala Arg	
480 485 490 495	
GAA ACA GAT GGA TAC TTC ATC AAG AGT GGG ATT GTC ACC GTC ATC AAG	1535
Glu Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Thr Val Ile Lys	
500 505 510	
GAT GCT TTG ATT CCA AGT GGA ATC ATC ATC TGATGAGCTC	1575
Asp Ala Leu Ile Pro Ser Gly Ile Ile Ile	
515 520	

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 521 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ala	Ala	Ser	Ile	Gly	Ala	Leu	Lys	Ser	Ser	Pro	Ser	Ser	Asn	Asn
1				5				10						15	
Cys	Ile	Asn	Glu	Arg	Arg	Asn	Asp	Ser	Thr	Arg	Ala	Val	Ser	Ser	Arg
		20					25						30		
Asn	Leu	Ser	Phe	Ser	Ser	Ser	His	Leu	Ala	Gly	Asp	Lys	Leu	Met	Pro
		35					40					45			
Val	Ser	Ser	Leu	Arg	Ser	Gln	Gly	Val	Arg	Phe	Asn	Val	Arg	Arg	Ser
	50					55				60					
Pro	Met	Ile	Val	Ser	Pro	Lys	Ala	Val	Ser	Asp	Ser	Gln	Asn	Ser	Gln
	65					70				75				80	
Thr	Cys	Leu	Asp	Pro	Asp	Ala	Ser	Arg	Ser	Val	Leu	Gly	Ile	Ile	Leu
			85					90					95		
Gly	Gly	Gly	Ala	Gly	Thr	Arg	Leu	Tyr	Pro	Leu	Thr	Lys	Lys	Arg	Ala
			100					105					110		

-51-

Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro
 115 120 125
 Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu Thr
 130 135 140
 Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Ala
 145 150 155 160
 Ser Asn Met Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val Leu Ala
 165 170 175
 Ala Gln Gln Ser Pro Glu Asn Pro Asp Trp Phe Gln Gly Thr Ala Asp
 180 185 190
 Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Thr Val Leu Glu
 195 200 205
 Tyr Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu Lys
 210 215 220
 Phe Ile Gln Ala His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala Ala
 225 230 235 240
 Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met Lys Ile
 245 250 255
 Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln Gly Glu
 260 265 270
 Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu Asp Asp
 275 280 285
 Lys Arg Ala Lys Glu Met Pro Phe Ile Ala Ser Met Gly Ile Tyr Val
 290 295 300
 Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe Pro Gly
 305 310 315 320
 Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser Leu Gly
 325 330 335
 Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly
 340 345 350
 Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys Lys Pro
 355 360 365
 Val Pro Asp Phe Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln
 370 375 380
 Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val Thr Asp
 385 390 395 400
 Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His His
 405 410 415

-52-

Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile Glu
420 425 430

Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala Asp Arg
435 440 445

Lys Leu Leu Ala Ala Lys Gly Ser Val Pro Ile Gly Ile Gly Lys Asn
450 455 460

Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Asp
465 470 475 480

Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala Arg Glu
485 490 495

Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Thr Val Ile Lys Asp
500 505 510

Ala Leu Ile Pro Ser Gly Ile Ile Ile
515 520

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1519 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1410

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAC AAG ATC AAA CCT GGG GTT GCT TAC TCT GTG ATC ACT ACT GAA AAT	48
Asn Lys Ile Lys Pro Gly Val Ala Tyr Ser Val Ile Thr Thr Glu Asn	
1 5 10 15	
GAC ACA CAG ACT GTG TTC GTA GAT ATG CCA CGT CTT GAG AGA CGC CGG	96
Asp Thr Gln Thr Val Phe Val Asp Met Pro Arg Leu Glu Arg Arg Arg	
20 25 30	
GCA AAT CCA AAG GAT GTG GCT GCA GTC ATA CTG GGA GGA GGA GAA GGG	144
Ala Asn Pro Lys Asp Val Ala Ala Val Ile Leu Gly Gly Gly Glu Gly	
35 40 45	
ACC AAG TTA TTC CCA CTT ACA AGT AGA ACT GCA ACC CCT GCT TT CCG	192
Thr Lys Leu Phe Pro Leu Thr Ser Arg Thr Ala Thr Pro Ala Val Pro	
50 55 60	

-53-

GTT GGA GGA TGC TAC AGG CTA ATA GAC ATC CCA ATG AGC AAC TGT ATC	240
Val Gly Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Ile	
65 70 75 80	
AAC AGT GCT ATT AAC AAG ATT TTT GTG CTG ACA CAG TAC AAT TCT GCT	288
Asn Ser Ala Ile Asn Lys Ile Phe Val Leu Thr Gln Tyr Asn Ser Ala	
85 90 95	
CCC CTG AAT CGT CAC ATT GCT CGA ACA TAT TTT GGC AAT GGT GTG AGC	336
Pro Leu Asn Arg His Ile Ala Arg Thr Tyr Phe Gly Asn Gly Val Ser	
100 105 110	
TTT GGA GAT GGA TTT GTC GAG GTA CTA GCT GCA ACT CAG ACA CCC GGG	384
Phe Gly Asp Gly Phe Val Glu Val Leu Ala Ala Thr Gln Thr Pro Gly	
115 120 125	
GAA GCA GGA AAA AAA TGG TTT CAA GGA ACA GCA GAT GCT GTT AGA AAA	432
Glu Ala Gly Lys Lys Trp Phe Gln Gly Thr Ala Asp Ala Val Arg Lys	
130 135 140	
TTT ATA TGG GTT TTT GAG GAC GCT AAG AAC AAG AAT ATT GAA AAT ATC	480
Phe Ile Trp Val Phe Glu Asp Ala Lys Asn Lys Asn Ile Glu Asn Ile	
145 150 155 160	
GTT GTA CTA TCT GGG GAT CAT CTT TAT AGG ATG GAT TAT ATG GAG TTG	528
Val Val Leu Ser Gly Asp His Leu Tyr Arg Met Asp Tyr Met Glu Leu	
165 170 175	
GTG CAG AAC CAT ATT GAC AGG AAT GCT GAT ATT ACT CTT TCA TGT GCA	576
Val Gln Asn His Ile Asp Arg Asn Ala Asp Ile Thr Leu Ser Cys Ala	
180 185 190	
CCA GCT GAG GAC AGC CGA GCA TCA GAT TTT GGG CTG GTC AAG ATT GAC	624
Pro Ala Glu Asp Ser Arg Ala Ser Asp Phe Gly Leu Val Lys Ile Asp	
195 200 205	
AGC AGA GGC AGA GTA GTC CAG TTT GCT GAA AAA CCA AAA GGT TTT GAT	672
Ser Arg Gly Arg Val Val Gln Phe Ala Glu Lys Pro Lys Gly Phe Asp	
210 215 220	
CTT AAA GCA ATG CAA GTA GAT ACT ACT CTT GTT GGA TTA TCT CCA CAA	720
Leu Lys Ala Met Gln Val Asp Thr Thr Leu Val Gly Leu Ser Pro Gln	
225 230 235 240	
GAT GCG AAG AAA TCC CCC TAT ATT GCT TCA ATG GGA GTT TAT GTA TTC	768
Asp Ala Lys Lys Ser Pro Tyr Ile Ala Ser Met Gly Val Tyr Val Phe	
245 250 255	
AAG ACA GAT GTA TTG TTG AAG CTC TTG AAA TGG AGC TAT CCC ACT TCT	816
Lys Thr Asp Val Leu Leu Lys Leu Lys Trp Ser Tyr Pro Thr Ser	
260 265 270	
AAT GAT TTT GGC TCT GAA ATT ATA CCA GCA GCT ATT GAC GAT TAC AAT	864
Asn Asp Phe Gly Ser Glu Ile Ile Pro Ala Ala Ile Asp Asp Tyr Asn	
275 280 285	

-54-

GTC CAA GCA TAC ATT TTC AAA GAC TAT TGG GAA GAC ATT GGA ACA ATT	912
Val Gln Ala Tyr Ile Phe Lys Asp Tyr Trp Glu Asp Ile Gly Thr Ile	
290 295 300	
AAA TCG TTT TAT AAT GCT AGC TTG GCA CTC ACA CAA GAG TTT CCA GAG	960
Lys Ser Phe Tyr Asn Ala Ser Leu Ala Leu Thr Gln Glu Phe Pro Glu	
305 310 315 320	
TTC CAA TTT TAC GAT CCA AAA ACA CCT TTT TAC ACA TCT CCT AGG TTC	1008
Phe Gln Phe Tyr Asp Pro Lys Thr Pro Phe Tyr Thr Ser Pro Arg Phe	
325 330 335	
CTT CCA CCA ACC AAG ATA GAC AAT TGC AAG ATT AAG GAT GCC ATA ATC	1056
Leu Pro Pro Thr Lys Ile Asp Asn Cys Lys Ile Lys Asp Ala Ile Ile	
340 345 350	
TCT CAT GGA TGT TTC TTG CGA GAT TGT TCT GTG GAA CAC TCC ATA GTG	1104
Ser His Gly Cys Phe Leu Arg Asp Cys Ser Val Glu His Ser Ile Val	
355 360 365	
GGT GAA AGA TCG CGC TTA GAT TGT GGT GTT GAA CTG AAG GAT ACT TTC	1152
Gly Glu Arg Ser Arg Leu Asp Cys Gly Val Glu Leu Lys Asp Thr Phe	
370 375 380	
ATG ATG GGA GCA GAC TAC TAC CAA ACA GAA TCT GAG ATT GCC TCC CTG	1200
Met Met Gly Ala Asp Tyr Tyr Gln Thr Glu Ser Glu Ile Ala Ser Leu	
385 390 395 400	
TTA GCA GAG GGG AAA GTA CCG ATT GGA ATT GGG GAA AAT ACA AAA ATA	1248
Leu Ala Glu Gly Lys Val Pro Ile Gly Ile Gly Glu Asn Thr Lys Ile	
405 410 415	
AGG AAA TGT ATC ATT GAC AAG AAC GCA AAG ATA GGA AAG AAT GTT TCA	1296
Arg Lys Cys Ile Ile Asp Lys Asn Ala Lys Ile Gly Lys Asn Val Ser	
420 425 430	
ATC ATA AAT AAA GAC GGT GTT CAA GAG GCA GAC CGA CCA GAG GAA GGA	1344
Ile Ile Asn Lys Asp Gly Val Gln Glu Ala Asp Arg Pro Glu Glu Gly	
435 440 445	
TTC TAC ATA CGA TCA GGG ATA ATC ATT ATA TTA GAG AAA GCC ACA ATT	1392
Phe Tyr Ile Arg Ser Gly Ile Ile Ile Ile Leu Glu Lys Ala Thr Ile	
450 455 460	
AGA GAT GGA ACA GTC ATC TGAAGTAGGG AAGCACCTCT TGTGAACTA	1440
Arg Asp Gly Thr Val Ile	
465 470	
CTGGAGATCC AAATCTCAAC TTGAAGAAGG TCAAGGGTGA TCCTAGCACG TTCACCAGTT	1500
GACTCCCCGA AGGAAGCTT	1519

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

-55-

(A) LENGTH: 470 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Asn Lys Ile Lys Pro Gly Val Ala Tyr Ser Val Ile Thr Thr Glu Asn
 1             5             10             15
Asp Thr Gln Thr Val Phe Val Asp Met Pro Arg Leu Glu Arg Arg Arg
      20             25             30
Ala Asn Pro Lys Asp Val Ala Ala Val Ile Leu Gly Gly Gly Glu Gly
      35             40             45
Thr Lys Leu Phe Pro Leu Thr Ser Arg Thr Ala Thr Pro Ala Val Pro
      50             55             60
Val Gly Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Ile
      65             70             75             80
Asn Ser Ala Ile Asn Lys Ile Phe Val Leu Thr Gln Tyr Asn Ser Ala
      85             90             95
Pro Leu Asn Arg His Ile Ala Arg Thr Tyr Phe Gly Asn Gly Val Ser
      100            105            110
Phe Gly Asp Gly Phe Val Glu Val Leu Ala Ala Thr Gln Thr Pro Gly
      115            120            125
Glu Ala Gly Lys Lys Trp Phe Gln Gly Thr Ala Asp Ala Val Arg Lys
      130            135            140
Phe Ile Trp Val Phe Glu Asp Ala Lys Asn Lys Asn Ile Glu Asn Ile
      145            150            155            160
Val Val Leu Ser Gly Asp His Leu Tyr Arg Met Asp Tyr Met Glu Leu
      165            170            175
Val Gln Asn His Ile Asp Arg Asn Ala Asp Ile Thr Leu Ser Cys Ala
      180            185            190
Pro Ala Glu Asp Ser Arg Ala Ser Asp Phe Gly Leu Val Lys Ile Asp
      195            200            205
Ser Arg Gly Arg Val Val Gln Phe Ala Glu Lys Pro Lys Gly Phe Asp
      210            215            220
Leu Lys Ala Met Gln Val Asp Thr Thr Leu Val Gly Leu Ser Pro Gln
      225            230            235            240
Asp Ala Lys Lys Ser Pro Tyr Ile Ala Ser Met Gly Val Tyr Val Phe
      245            250            255

```

-56-

Lys Thr Asp Val Leu Leu Lys Leu Leu Lys Trp Ser Tyr Pro Thr Ser
 260 265 270
 Asn Asp Phe Gly Ser Glu Ile Ile Pro Ala Ala Ile Asp Asp Tyr Asn
 275 280 285
 Val Gln Ala Tyr Ile Phe Lys Asp Tyr Trp Glu Asp Ile Gly Thr Ile
 290 295 300
 Lys Ser Phe Tyr Asn Ala Ser Leu Ala Leu Thr Gln Glu Phe Pro Glu
 305 310 315 320
 Phe Gln Phe Tyr Asp Pro Lys Thr Pro Phe Tyr Thr Ser Pro Arg Phe
 325 330 335
 Leu Pro Pro Thr Lys Ile Asp Asn Cys Lys Ile Lys Asp Ala Ile Ile
 340 345 350
 Ser His Gly Cys Phe Leu Arg Asp Cys Ser Val Glu His Ser Ile Val
 355 360 365
 Gly Glu Arg Ser Arg Leu Asp Cys Gly Val Glu Leu Lys Asp Thr Phe
 370 375 380
 Met Met Gly Ala Asp Tyr Tyr Gln Thr Glu Ser Glu Ile Ala Ser Leu
 385 390 395 400
 Leu Ala Glu Gly Lys Val Pro Ile Gly Ile Gly Glu Asn Thr Lys Ile
 405 410 415
 Arg Lys Cys Ile Ile Asp Lys Asn Ala Lys Ile Gly Lys Asn Val Ser
 420 425 430
 Ile Ile Asn Lys Asp Gly Val Gln Glu Ala Asp Arg Pro Glu Glu Gly
 435 440 445
 Phe Tyr Ile Arg Ser Gly Ile Ile Ile Ile Leu Glu Lys Ala Thr Ile
 450 455 460
 Arg Asp Gly Thr Val Ile
 465 470

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

-57-

GTTGATAACA AGATCTGTGA ACCATGGCGG CTTC

35

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCAGTTAAAA CGGAGCTCAT CAGATGATGA TTC

33

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTGTGAGAAC ATAAATCTTG GATATGTTAC

30

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAATTCACAG GGCCATGGCT CTAGACCC

28

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

-58-

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AAGATCAAAC CTGCCATGGC TTACTCTGTG ATCACTACTG

40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGAATTCAA GCTTGGATCC CGGGCCCCCC CCCCCCCC

39

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGAATTCAA GCTTGGATCC CGGG

24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCTCTAGACA GTCGATCAGG AGCAGATGTA CG

32

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAGTTAGCC ATGGTTAGTT TAGAG

25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCCGAGCTC GTCAACGCCG TCTGCGATT GTGC

34

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GATTTAGGTG AACTATAG

19

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

-60-

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGAGAGATCT AGAACAATGG CTCCTCTAT GCTCTCTTCC GC

42

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGCCGAGCTC TAGATTATCG CTCCTGTTTA TGCCCTAAC

39

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2196 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATCGATTATT GGTTCATCGG GTTTTGATCG TTATCGGTTT GGTTCACCG TTAAATTTG	60
ACACAAAAT AAAATTGAA AAGCACTTAG AAACAAGGTG ACAAACCTAA TAAACCATGC	120
ACATGAGTTC ACAAGTTACA TCTTGCTAAA AAACAAACAC TTTTACATTG TAGAATAACC	180
AAGTGTCTGG GACAACCAA AATGAAAGTA GGAAACCAA CTCTAAGTCA AGGACTTTAT	240
ATACAAAATG GTATAACTAT AATTATTTAA TTTACTATTG GGTTATCGGT TAACCCGTTA	300
AGAACCGATA ACCCGATAAC AAAACAATC AAAATCGTTA TCAAACCGC TAAACTAATA	360
ACCCAATACT GATAAACCAA TAACTTTTTT TTTATTCGGG TTATCGGTTT CAGTTCGGTT	420
TTGAACAATC CTAGTGCCT AATTATTGTT TTGAGAACCA AGAAAACAAA AACTGACGTC	480

GCAAATATTT CAGTAAATAC TTGTATATCT CAGTGATAAT TGATTTCCAA GATGTATAAT 540
TATCATTTAC GTAATAATAG ATGGTTTCCG AAACCTACGC TTCCCTTTTT TCTTTTGCAG 600
TCGTATGGAA TAAAGTTGGA TATGGAGGCA TTCCCGGGCC TTCAGGTGGA AGAGACGGAG 660
CTGCTTCACA AGGAGGGGGT TGTGTACTT GAAAATAGGC ATTTATTCCG TTCGCAAACC 720
TATCATGTTT CTATGGTTGT TTATTTGTAG TTTGGTGTTT TTAATATCGA GTGTTCTTTA 780
GTTTGTTCCT TTTAATGAAA GGATAATATC TCGTGCCAAA AATAAGCAAA TTCGGTACAT 840
AAAGACATTT TTTTCTTTT GTGGATTTT TGTTTATGGA GTTGTCAAAT GTGGAATTTA 900
TTTCATAGCA TGTGGAGTTT CCTCCTCTCC TTTTTCATGT GCCCTTGGGC CTTGCCTGTT 960
TCTTGACCCG CAGTGTGCCA GGGCAGTCGG CAGATGGACA TAAATGGCAC ACCGCTCGGC 1020
TCGTGGAAAG AGTATGGTCA GTTTCATTGA TAAGTATTTA CTCGTATTCG GCGTATACAT 1080
CAAGTTAATA GAAAGTAAAC ACATATGATA TCATACATCC ATTAGTTAAG TATAAATGCC 1140
AACTTTTTAC TTGAATCGCT GAATAAATTT ACTTACGATT AATATTTAGT TGTGTGTTCA 1200
AACATATCAT GCATTATTTG ATTAAGAATA AATAAACGAT GTGTAATTTG AAAACCAATT 1260
AGAAAAGAAG TATGACGGGA TTGATGTTCT GTGAAATCAC TGGCAAATTG AACGGACGAT 1320
GAAATTTGAT CGTCATTTAA ACATATCAAC ATGGCTTTAG TCATCATCAT TATGTTATAA 1380
TTATTTTCTT GAACTTGAT ACACCAACTC TCATTGGGAA AGTGACAGCA TAATATAAAC 1440
TATAATATCA ATCTGGCAAT TTCGAATTAT TCCAAATCTC TTTTGTCAAT TCATTTTCATC 1500
CCCTATGTCT GCCTGCAAGT ACCAATTATT TAAATACAAA AATCTTGATT AAACAATTCA 1560
TTTTCTCACT AATAATCACA TTTAATAATA AACGGTTCAT ACACGTGCGT CACCTTTTTT 1620
TCGATTTTCT CTCAAGCGCA TGTGATCATA TCTAACTCTT GTGCAAACAA GTGAAATGAC 1680
GTCCATTAAT AAATAATCTT TTGAATACCT GTTCATTTTA ATTTATTTGG ATTTGCTAAG 1740
GATTTTTTTT AGTTTTTGAG ATTTTTTATA ATTTTAAATT AAAAAAATA AGTTAAATAT 1800
ATCGAAAATG TCTTTTAATC TTATTTTGA AAAAGATAAT TAGCTCAAAC AAATTAAAT 1860
TGGTAACTAT TTTTCGGAAA AATAATGATT CTTATTGTAC ATTCTTTTTT ATCGATTAGA 1920
TATTTTTTTT AAGCTCAAGT ACAAAGTCA TATTTCATC CCCAAAATAG CCTCAATCAC 1980
AAGAAATGCT TAAATCCCCA AAATACCCTC AATCACAAAA AGTGATACCA TCATAACTAT 2040
GGTCCTCTGT AAATTCCAAC AAAATCAAGT CTATAAAGTT ACCCTTGATA TCAGTACTAT 2100
AAAACCAAAA ATCTCAGCTG TAATTCAAGT GCAATCACAC TCTACCACAC ACTCTCTAGT 2160

AGAGAAATCA GTTGATAACA AGCTTTGTTA ACAATG

2196

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGCTTGATA TCGAATTCCT GCAGCCCGGG GGATCTCCTT AAAACTTTTT CTGAATTACT	60
TTTCAAGATT CTTGATTCTG CACCACTAGC AATTTCCATT TTTCTTTCAG TGATTTTGGT	120
TACTTATTTG ACATTCTTGT TTTCAAGATC CAACATCATC ACTTTCAGG TTCAAAATCT	180
TGTTTTTTTT CTTTTTCTT TTAATGCTCT ATATTGTGGA AGTCCACAGG TGAATTTTTA	240
CGATATGGGT TTACCACTTA GCTTCTTGT AATATTTTAT CAATTTTAGA AAATATATGT	300
GTGAAATACC TAATTTTACG TAGAGATCAT GGGTTCATAT GCGTAAAGAT TCATGTTTTT	360
GTGGTAATGC TATGAGGTAT TAGTACTGAG CATATAGCTA GCTTGGGTTT TGGGTTTACC	420
GACCAAAAAA AAAAATTAGT GATATTTTCT TTATGTAAAT TATACTTTTC TTGGTTGCTA	480
AAAGATAACA TATACTTTAT TGAGATTTGA ATAAATCTAT TTGATTTAGA TCCATTGATA	540
AATCTTAATC TTATGGGATT ACTGATTTGT TGATTGGCTG CAGAAGGATC C	591

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1705 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGCTTGGGT ACCGGGCCCC CCCTGGAGGT CGAGTGCCAT CACATCCAGG GTGTAGGCTC	60
GGGGCGTGAC AACTTGGTA TCTGAGCTCA GAGTTCAAGA GTCTAAGGTG TCTATAAAGT	120
CTGTGCCTTT AGAGTCCTAG TTATCGGTGT GAAGCGCGCC ACATCTATAA CCAGGAGGCT	180

GCGACATTTA AGAATTATCA TACTTCTTTC ATACTCTTTT CGTGCAATAG AGTTCAACTC	240
CATAAAGTCT CTTTATAATT CATGTTTACG CATATCTTTG AGATCATGCC TCCATGTAGA	300
GTTGTCTGAG GTCGTCCTGC TAGAAGAAAT ATTGATCCTC AGGATCAAGG GGTACCCAAAT	360
GCACCAGAAG TGCGACCCCA AGGAAAGGTC ACTAATGTTG AGTTCCAGGA TGTTATACGG	420
ATATTGAGTG AAGTTGTGAC CAACCAAGCT GGACAACAAA GAGGGAATCA ACAAGATGTG	480
GTTGATACAT CCAGAATCCG TGAGTTCTTA AGGATGAATC CTCAGACTT CACCAATTCA	540
AGAGTCACTG AGGATCTGGA AAACCTTGTG GAAGAGTTGT AGAAGGTTTT TGAGGTTATG	600
CATGTTGTTG ATGCTGAGCG AGTGGAACCTA ACTGCATACC AACTGAATGG TGTGCTAGA	660
GTATGGTACG ACCAATAGAA AAAGAGTAGA GTTGAGGGTG CACAAATTGT GAGTTGGGCA	720
GTGTTTGAAG AGGCCTTCAT GGGGCATTTT TTTTCCCATG AACTATATGG CAAAGGTAAG	780
AGAATTTCTT CACTCTTAAG CAGGAATCCA TGAGTGTGCA TAAGTATAGC CTCAGTTCA	840
CTCAACTGTC GCCTATGCTC CAGAGATGGC TGTTGATATG AGGAGCAGGA TGGGCTTGTT	900
TGTGTTTGGG TTGTCTCATC TGTCAATCAA AGAAGGTAAG GTTGTGATGT GGATAAAGGA	960
CATGGACATC GAAAGGGTAA TGATCCTTGT GCAACAGGTT GAGGAAGATA AGTTGAGGGA	1020
TAGAGAAGAG TTCTGAAACA AGAGGGCTAA GAACACATGA AATGAGTACG TAAGCAGAAG	1080
AGTAATGCAA ATCGGTTATC TTTTCAATGA AAGCCAAATA AACCTGCTTG ATTGTTTGCA	1140
AGTGCAACCT GTACCAACGA ACAAAGGTGA GTTCAAGAAT CAGAATTCTT AGAAATTCAG	1200
AGCTAGACCT GCACAATCTC AAGGTAGTGT GGCACAAGGA TGTAATGGGA CTCCTGCATG	1260
TGTTAAGTAC GGTAGGAACC ACCCAGGAGC GTGTCATGAT GGCTCTGCTG GTTGCTTCAA	1320
GTGTGGTCAG AATGGTCACT TCATGAGAGA GTGCCTAAAG AANAGGCAAG GTAATAGCAA	1380
TGGGGGCAAT ATATCACAAT CTTCTTCAGT GGCTCCACNA GATAGAGCTG CACCTTGAGG	1440
ATCATGGGTT CATATGCGTA AAGATTCATG TTTTGTGGTA ATGCTATGAG GTATTAGTAC	1500
TGAGCATATA GCTAGCTTGG GTTTTGGGTT TACCGACCAT TTTTTTTAAT TAGTGATATT	1560
TTCTTTATGT ATTTTATACT TTTCTTGGTT GCTTAAAGAT TACATATACT TTATTGAGAT	1620
TTGAATAAAT CTATTTGATT TAGATCCATT GATAAATCTT AATCTTATGG GATTACTGAT	1680
TTGTTGATTG GCTGCAGAAG GATCC	1705

Claims:

1. A method of improving the quality of potato tubers stored at reduced temperatures comprising providing an increased level of ADPglucose pyrophosphorylase enzyme activity within the tuber during storage at reduced
5 temperatures by transforming potato plants with a recombinant, double-stranded DNA molecule comprising (a) a promoter which functions in potatoes to cause the production of an RNA sequence in tubers, (b) a structural DNA sequence that causes the production of an RNA sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an
10 ADPglucose pyrophosphorylase enzyme, and (c) a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence; and obtaining tubers from such transformed potato plants, wherein said ADPglucose pyrophosphorylase enzyme is deregulated.

15

2. The method of Claim 1 wherein said ADPglucose pyrophosphorylase enzyme is the *E. coli glgC* enzyme.

3. The method of Claim 1 wherein said ADPglucose
20 pyrophosphorylase enzyme is a mutant *E. coli* enzyme.

4. The method of Claim 3 wherein said ADPglucose pyrophosphorylase enzyme has the sequence shown in SEQ ID NO:4.

25 5. A method of reducing the level of sugars within potato tubers stored at reduced temperatures comprising providing an increased level of ADPglucose pyrophosphorylase enzyme activity during storage at reduced temperatures by transforming potato plants with a recombinant, double-stranded DNA molecule comprising (a) a promoter which functions in potatoes
30 to cause the production of an RNA sequence in tubers, (b) a structural DNA sequence that causes the production of an RNA sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an ADPglucose pyrophosphorylase enzyme, and (c) a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination
35 and the addition of polyadenylated nucleotides to the 3' end of the RNA

-65-

sequence; and obtaining tubers from such transformed potato plants, wherein said ADPglucose pyrophosphorylase enzyme is deregulated.

6. The method of Claim 5 wherein said ADPGPP enzyme is the *E. coli glgC* enzyme.

7. The method of Claim 5 wherein said ADPGPP enzyme is a mutant *E. coli* enzyme.

8. The method of Claim 7 wherein said ADPGPP enzyme has the sequence shown in SEQ ID NO:4.

9. A recombinant, double-stranded DNA molecule comprising in operative sequence:

- (a) a cold-inducible promoter which functions in plants to cause the production of an RNA sequence in target plant tissues,
 - (b) a structural DNA sequence that causes the production of an RNA sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an ADPglucose pyrophosphorylase enzyme, and
 - (c) a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence;
- wherein said ADPglucose pyrophosphorylase enzyme is deregulated.

10. The DNA molecule of Claim 9 wherein said ADPglucose pyrophosphorylase enzyme is the *E. coli glgC* enzyme.

11. The DNA molecule of Claim 9 wherein said ADPglucose pyrophosphorylase enzyme is a mutant *E. coli* enzyme.

12. The DNA molecule of Claim 11 wherein said ADPglucose pyrophosphorylase enzyme has the sequence shown in SEQ ID NO:4.

13. The DNA molecule of Claim 9 wherein said promoter is from

-66-

potato.

14. A potato plant cell comprising a recombinant, double-stranded DNA molecule comprising in operative sequence:

- 5 (a) a cold-inducible promoter which functions in plants to cause the production of an RNA sequence in target plant tissues,
- (b) a structural DNA sequence that causes the production of an RNA sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an ADPglucose
- 10 pyrophosphorylase enzyme, and
- (c) a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence;
- wherein said ADPglucose pyrophosphorylase enzyme is deregulated.

15

15. The potato plant cell of Claim 14 wherein said ADPglucose pyrophosphorylase enzyme is the *E. coli glgC* enzyme.

16. The potato plant cell of Claim 14 wherein said ADPglucose

20 pyrophosphorylase enzyme is a mutant *E. coli* enzyme.

17. The potato plant cell of Claim 16 wherein said ADPglucose pyrophosphorylase enzyme has the sequence shown in SEQ ID NO:4.

25 18. The potato plant cell of Claim 14 wherein said promoter is from potato.

19. A potato plant consisting of cells of Claim 14.

30 20. The potato plant of Claim 19 wherein said ADPglucose pyrophosphorylase enzyme is the *E. coli glgC* enzyme.

21. The potato plant of Claim 19 wherein said ADPglucose pyrophosphorylase enzyme is a mutant *E. coli* enzyme.

35

-67-

22. The potato plant of Claim 21 wherein said ADPglucose pyrophosphorylase enzyme has the sequence shown in SEQ ID NO:4.

23. The potato plant of Claim 19 wherein said promoter is from 5 potato.

24. A method of prolonging dormancy of stored potato tubers comprising providing an increased level of ADPglucose pyrophosphorylase enzyme activity within the tuber during storage by transforming potato plants 10 with a recombinant, double-stranded DNA molecule comprising (a) a promoter which functions in potatoes to cause the production of an RNA sequence in tubers, (b) a structural DNA sequence that causes the production of an RNA sequence which encodes a fusion polypeptide comprising an amino-terminal plastid transit peptide and an ADPglucose pyrophosphorylase enzyme, and (c) 15 a 3' nontranslated DNA sequence which functions in plant cells to cause transcriptional termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence; and obtaining tubers from such transformed potato plants, wherein said ADPglucose pyrophosphorylase enzyme is deregulated.

20

25. The method of Claim 24 wherein said storage is at reduced temperatures.

26. The method of Claim 25 wherein said ADPGPP enzyme is the 25 *E. coli glgC* enzyme under the control of a cold-inducible promoter.

27. The method of Claim 25 wherein said ADPGPP enzyme is a mutant *E. coli* enzyme under the control of a cold-inducible promoter.

30 28. The method of Claim 27 wherein said ADPGPP enzyme has the sequence shown in SEQ ID NO:4.

29. The method of Claim 24 wherein said tubers are from plants of Claim 19.

35

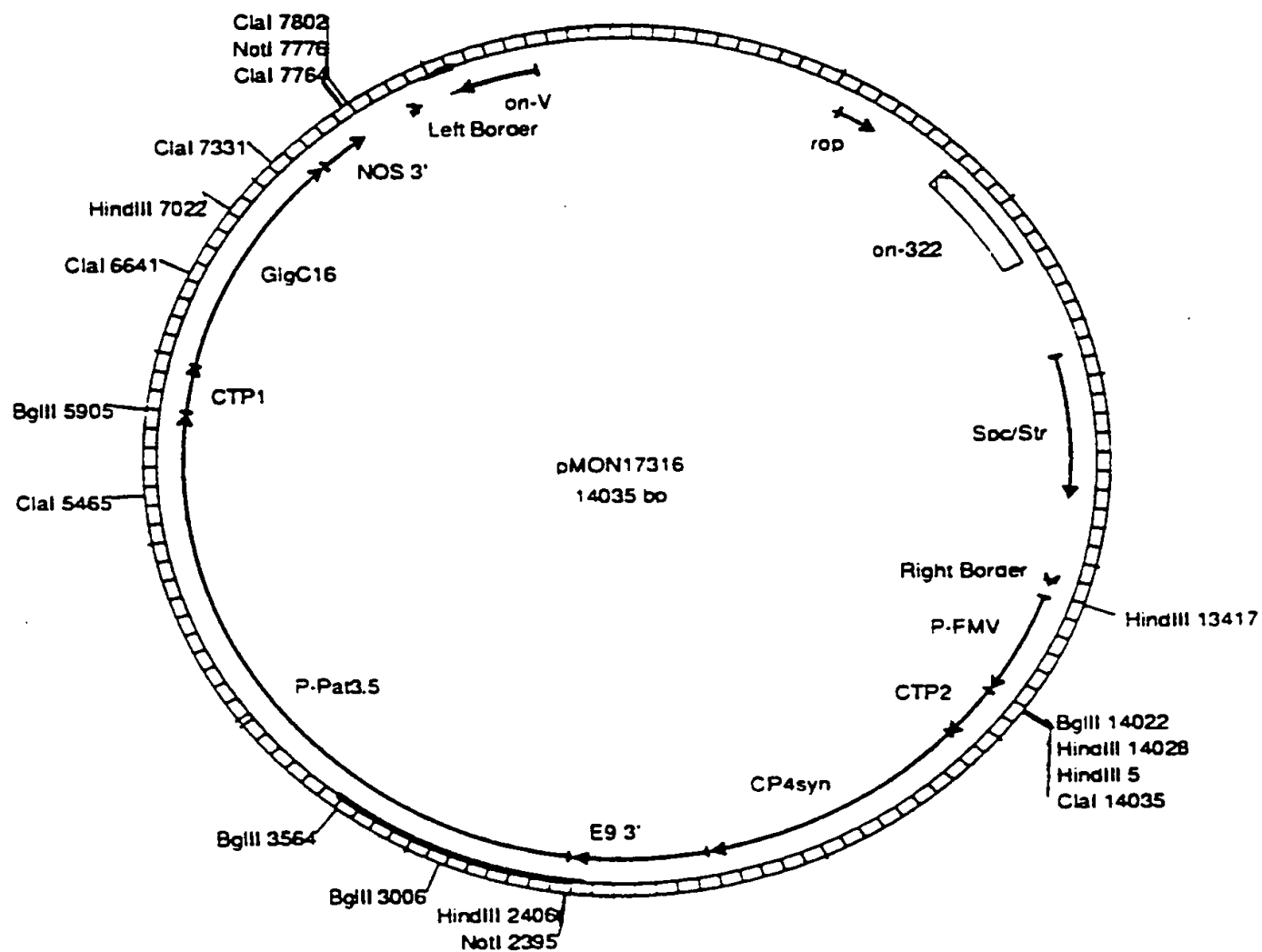


FIGURE 1

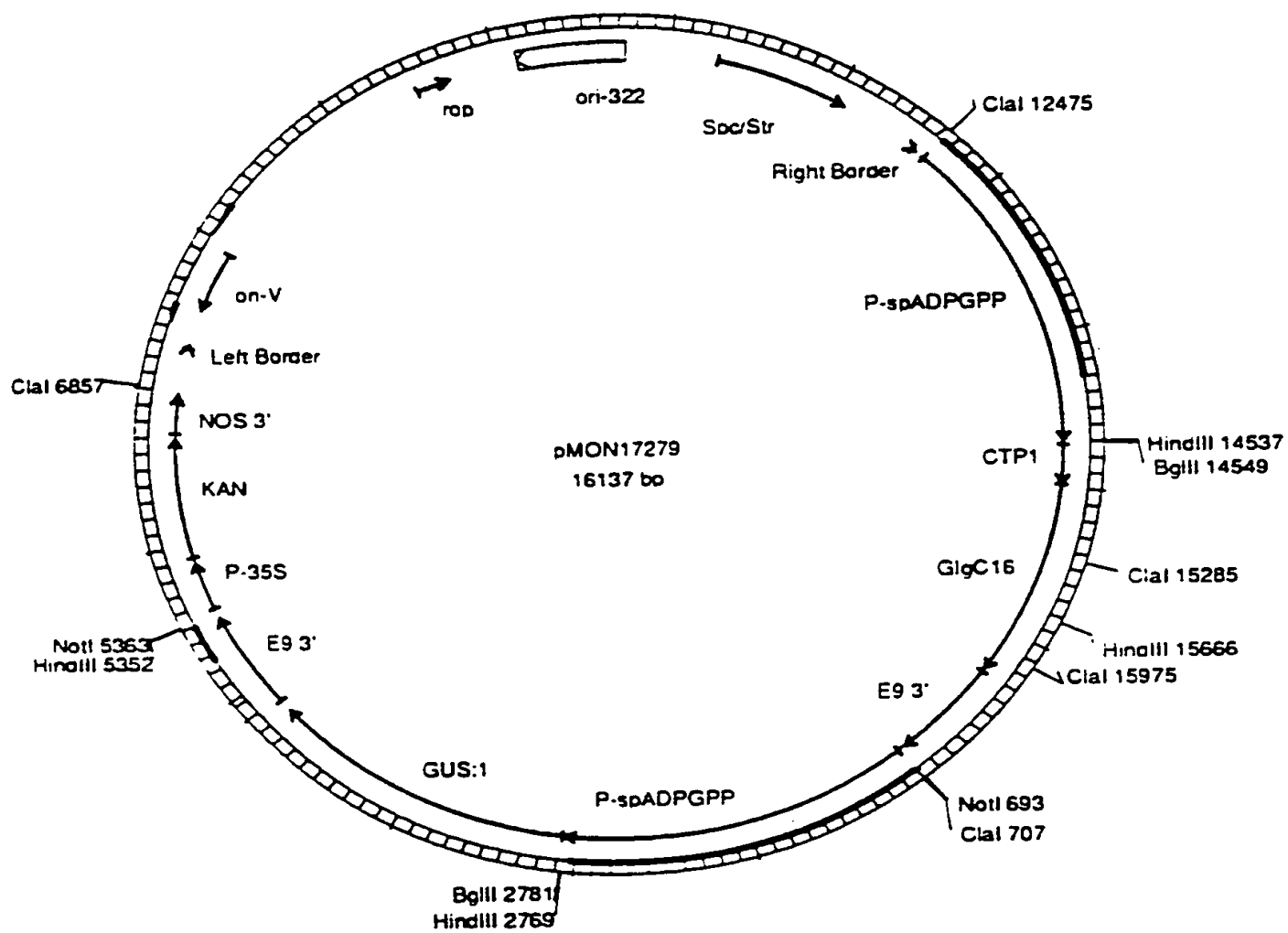


FIGURE 2

INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 94/05275

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/82 C12N15/54 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 438 904 (ADVANCED TECHNOLOGIES) 31 July 1991 cited in the application see the whole document ---	1-29
A	WO,A,91 19806 (MONSANTO) 26 December 1991 cited in the application see the whole document -----	1-29

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* "&" document member of the same patent family

Date of the actual completion of the international search

26 September 1994

Date of mailing of the international search report

18. 10 94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/05275

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0438904	31-07-91	AU-A- 6836590 JP-A- 4341126	04-07-91 27-11-92
WO-A-9119806	26-12-91	AU-B- 644203 AU-A- 8220291 EP-A- 0536293	02-12-93 07-01-92 14-04-93

